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SER. II, VOL. 12 *March, 1950* No. 5

TRANSACTIONS
OF
THE NEW YORK ACADEMY OF SCIENCES

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Contents

Composition of Some New England Granites. *By* FELIX CHAYES 144
The Search for Long Range Forces in Solution. *By* BENJAMIN CARROLL 152
The Use of Neotetrazolium as a Tool in the Study of Active Cell Processes. *By* WILLIAM ANTOPOL, SUSI GLAUBACH, AND LESTER GOLDMAN 156
The Use of Neotetrazolium in the Study of *E. coli* Metabolism. *By* HIROMICHI T. NARAHARA, HOWARD QUITTNER, LESTER GOLDMAN, AND WILLIAM ANTOPOL 160
Influence of Tetrazolium Salt on the Growth and Cytology of Root Tips. *By* B. P. SONNENBLICK, W. ANTOPOL, AND L. GOLDMAN 161
An Introduction to the Work on Extrasensory Perception. *By* J. B. RHINE 164
Isolation of Culturable Fungi from Diverse Habitats. *By* B. M. DUGGAR 168
Archaeological Research in Afghanistan. *By* WALTER A. FAIRSERVIS, JR. 172
Conference Held: "The Growth, Replacement, and Types of Hair" 174
New Members 176

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TRANSACTIONS of The New York Academy of Sciences, Series II, Volume 12, No. 5, March, 1950.
Entered as second-class matter December 12, 1947, at the post office at Baltimore, Maryland, under the act of August 24, 1912. This publication is distributed to members and is published monthly from November to June, inclusive, by The New York Academy of Sciences, Mt. Royal and Guilford Aves., Baltimore 2, Md. Editorial office: 2 East Sixty-third Street, New York 21, N. Y.

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COMPOSITION OF SOME NEW ENGLAND GRANITES*

By FELIX CHAYES†

The Present Status of Descriptive Petrography. The science of descriptive petrography has changed very little during the past sixty years. In the course of less than a generation it was brought from oblivion to a stage at which further progress was possible only along quantitative lines, and that is about where it is today.

For most of this development, we are indebted to the industry and skill of perhaps a half dozen men. The remarkable edifice they constructed still stands, and we still live in it. If our interest is in the qualitative aspects of description, then wherever we turn, we shall find we have been anticipated. We cannot hope to stand still indefinitely and must soon find answers to two questions. In the first place, could we, under any circumstances, develop a form of petrographic description related to conventional petrography in the way that quantitative analysis is related to qualitative analysis in chemistry? In the second place, should we do so?

These questions are by no means identical. An enormous amount of time and labor will be consumed in getting any kind of answer for the first. If the answer to the second is "No," all this time and labor will be lost. Thus, the second question is of paramount importance, and our answer to it must not be dictated by mere exhibitionism or a desire to outdo our grandfathers. Many things they did we do not have to do again. Jumping down out of trees was once a major undertaking for the whole race, or its precursors; today it is only a sport for small boys who are lucky enough to live where there are trees. In our day, a new demonstration of the organic origin of fossils would be an achievement of very modest dimensions. Unimpeachable quantitative evidence that granites contain less biotite than quartz would fall in the same category.

It is the author's opinion that a systematic quantitative petrography would be extraordinarily useful and that its development is entirely feasible. The present paper will concentrate on this question of feasibility and argue, first very generally and then by a specific example, that the availability of reliable quantitative modal analyses in sufficient number would have immediate and profound effect on petrological theory.

The Debate about the Origin of Granite. The modern science of petrology owes its birth to a bitter controversy over the origin of granite and, in one way or another, this question has always been before us. Since 1930, the discussion has become progressively less scientific and more personal and, last year, it began to go political. Magmatists, according to Perrin and Roubault,[1] are conservatives, while antimagmatists range through the remainder of the political spectrum to Reed, who is pink, and Perrin and Roubault, who are both redder than the rose. Of course, these two are not only extreme revolutionaries but devout liberals as well. The whole patter has a familiar, hollow ring. The present situation in genetics is a splendid example of its consequence.

Now, it is not hard to put the core of the granite dilemma in very simple terms. Must we suppose the existence and movement of large quantities of liquids (magmas) of granitic composition in the upper portions of the lithosphere at times and in places where there is little or no evidence that these liquids have pierced the crust and been discharged as extrusives?

The question is stated here as it might be put by a magmatist trying hard to be fair, for the magmatist is of course impressed by

* This paper was presented at the meeting of the Section, February 6, 1950.

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vast volumes of lavas of nongranitic composition whose existence and survival in the lithosphere *as liquids* no one seriously doubts. He is also inclined to regard the smaller, but still very respectable, volume of tuffs and extrusives of granitic composition as so much granite magma that has escaped from the crust. He thus feels no doubt that the key assumption is permissible, and the only question he will admit is whether it is necessary. If it is shown to be necessary, even the antimagmatist may ultimately give in.

The antimagmatist, also trying to be fair, will probably insist—in fact, has insisted many times—that even this moderate statement of the issue reveals a latent magmatic bias. He is not prepared to discuss the necessity of the assumption until he is persuaded that it is permissible. If he can show that it is not permissible, the question of its necessity becomes academic.

Now, all this fanfare about protocol would pose a pretty problem indeed if geology were a branch of logic; it might be that we could never discuss the matter sensibly. In a natural situation as complex as this, however, the distinction between necessary and permissive hypotheses is far from sharp. What we really want is a useful hypothesis, or a hypothesis that is more useful than others. If it works, it is surely permissible; and no matter how well it works we can never be entirely sure it is necessary.

Concerning the composition of individual granite masses, or of groups of granites, classical petrography (petrography as we now practice it) never seems to generate the kind of evidence which would permit a firm and generally acceptable resolution of the controversy. If it can once swallow the curious fact that all granite masses are very similar in average or bulk composition, then, as far as composition is concerned, any hypothesis at all survives. In discussions of the origin of granite, the composition of granite thus plays at most a minor role. In fact, its very unimportance is sometimes cited as evidence of the strength of one or another hypothesis. Proponents of several of the transformist hypotheses openly boast that a granite can be made out of anything at all and offer no specification about the mineralogy of the finished product save that it is on the whole "granitic in composition."

I think we must regard this situation as one of the major failures of classical petrography, for the composition and variation in composition of a granite mass are among its most important properties. Indifference about major characteristics is never an indication of the strength of a theory. At best it reflects an appalling weakness in the data which the theory attempts to rationalize.

It is possible, though intuitively we can hardly consider it very probable, that hypotheses as different as crystallization from a liquid (with or without fractionation), crustal melting (with or without accumulation of the melt in a separate reservoir prior to recrystallization), solid diffusion, and the various forms of syntectic or migmatic processes would all yield bodies of the same average or bulk composition. With regard to internal variations in the composition of each mass, however, the assumption of an initially homogeneous parent material leads to inferences diametrically opposed to those drawn from other hypotheses. Reliable quantitative information about the internal variability of granite masses might thus go a long way toward settling the problem. For the magmatic hypotheses do in general contain the assumption of a homogeneous parent, while this assumption is conspicuously lacking in the nonmagmatic hypotheses, and its absence is usually intentional.

The Quartz Content of Some New England Granites. In order to keep the discussion within reasonable bounds, certain matters that ought to be treated very fully must be accepted on faith. In the first place, all the results used here are thin-section analyses made by point counter.* The analyt-

* In this procedure, a symmetrical point grid is cast over the area whose composition is sought, material underlying each point is identified, and the total frequencies recorded are reduced to percentages. For a description of the technique see F. Chayes, Amer. Min. **34**: 1–11, 1949.

ical error is on the order of 1 per cent for major constituents and less than this for minor ones. Each analysis is of an area not less than ¾ nor more than 1 inch square. The analyses are all the author's own work. They perhaps reflect some personal bias in identification, but it is felt that this is at a minimum. The advantage of having all measurements by one man is that interoperator differences are eliminated. In the present state of the subject, these differences are a highly significant source of variation, in many cases, much more important than differences between rocks.

Each thin section has been cut from a different hand specimen, and, with one exception, the hand specimens were never taken from sites less than fifty feet apart. Some are from different parts (usually different working faces) of the same quarry, but most are from different quarries. The quarries occur in irregular clusters in a roughly triangular area, bounded on the west by a line connecting Westerly, Rhode Island, and Woodbury, Vermont, and extending east to North Sullivan, Maine, on the north shore of Frenchmans Bay.* Nineteen quarry areas are represented in all, but from each of five areas fewer than five specimens were taken. In the others, the sample size ranged from seven to twenty-two, varying roughly with the number and size of quarries. It is with these that the discussion is chiefly concerned, though nothing in the smaller samples contradicts any of the findings. The sample is entirely confined to granites or granodiorites described by Dale[2] as other than coarse- or medium coarse-grained. It includes specimens from all the major commercial bodies of this type and is, I believe, by far the best sample of the finer-grained calc-alkaline granites of New England, so far subjected to quantitative modal analysis.

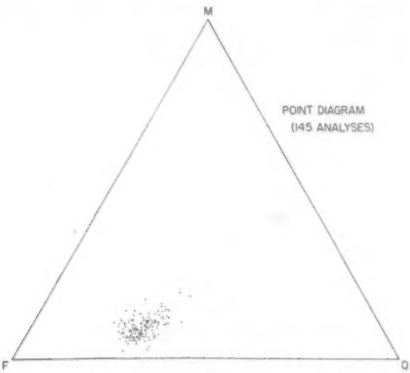

FIGURE 1. Point diagram of volume per cent of quartz (Q), total feldspar (F), and mafic minerals (M) in 145 thin sections of New England granite.

Full details of sampling and analytical techniques, together with a complete table of analyses, will be ready for publication soon. For the present discussion, which is primarily intended to show that data of this type would be useful if we had them, the existence and application of adequate sampling and analytical techniques will have to be taken for granted. Finally, the place of a complete table of analyses will be taken by ternary diagrams of two types, both of which will be familiar to the reader.

The first series of projections is of the constituents Total Feldspar (F)—Quartz (Q)—mafic minerals (M), where M includes biotite, muscovite, opaque, and transparent accessories. The results for each granite are very similar and a summary of the total data is given in FIGURES 1 and 2. FIGURE 1 is a scatter diagram for the whole suite of 145 thin sections. Despite the close concentration shown on the point diagram, it is often a comparatively simple matter to distinguish these granites from each other on the basis of quartz content or color index. If space permitted an examination of the analyses instead of the diagrams, it would become evident that thin-section analysis is a technique far more powerful than is generally suspected.

For our immediate purpose, however, the remarkable similarity of all these results is much more important. FIGURE 2 is a contour diagram prepared from FIGURE 1 in the fashion common in petrofabrics except

* Westerly is a little over 200 miles south and North Sullivan a little over 200 miles east of Woodbury.

that a hexagon of 0.12 per cent area was used in place of a circle of 1 per cent. Notice that, even with this very small counter area, a 9 per cent maximum appears on the diagram. The 9 per cent contour encloses 0.5 per cent of the total area of the triangle and contains 47 per cent of the results. The total occupied area of the diagram is only 4 per cent. Concentrations of this order are unknown in petrofabrics; apparently even poles of mica cleavages in schist never approach it.

Now, if each point on the scatter diagram were in some sense an average value or an estimate of the bulk composition of a considerable mass of rock, we would be back just about where we started. The average composition of granite has been public knowledge for a matter of fifty years or so, and in some fashion every hypothesis manages to account for or ignore it. To some extent, too, the agreement about average or bulk composition is a matter of definition.

It is worth stressing again, therefore, that the points in FIGURE 1 are in no sense either averages or estimates of bulk composition. Each point is a plot of the result of a single analysis of a single thin section less than 1 inch in area. So far as it represents any real quantity of rock at all, the relevant volume is not that of a stock or a quarry or even a hand specimen; it is, at most, the volume of a single thin section, perhaps 1/1000 of a cubic inch. This absurdly small sample is taken in the worst possible fashion, with no sifting, rolling, or homogenizing of any sort. What is more, no two thin sections are from the same hand specimen, most of the hand specimens are from different quarries, and the quarries are widely scattered over New England. We are here in the presence of one of petrology's major mysteries, a mystery no serious hypothesis can afford to scoff at or fail to rationalize.

At this point, we may examine the assertion, made toward the close of the last section, that hypotheses based on the assumption of a homogeneous parent material for granite would give rise to inferences about internal variations very different from those generated by hypotheses from which this assumption was lacking.

If each granite was formed by undisturbed crystallization of a liquid of about the same composition as the finished product, it should occasion no surprise that each granite mass exhibits slight internal variation. With regard to color index, quartz, and total feldspar content, this proves to be the case. If the liquids differed in composition, we ought to be able to detect this difference, no matter how slight, by the use of large enough samples of each

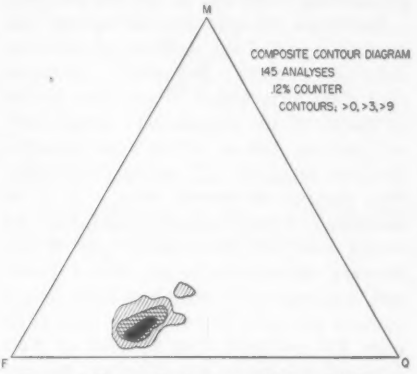

FIGURE 2. Contour diagram prepared from point diagram of FIGURE 1.

granite. The size of sample required for this purpose will vary with both intergranite differences and intragranite variation. Now, the existence of intergranite differences is easily detected by formal analysis of the data of FIGURE 1, and, as neither the sample size nor the total range of variation is large, we must conclude that the internal variability is very small indeed. It is, I believe, far smaller than the most sanguine magmatist would have anticipated. Evidently, we may not conclude either that there is a *single* granite magma, or that all granite magmas are of exactly the same composition. This complicates the matter, of course, but has nothing to do with the question of whether granite magmas exist.

According to nonmagmatic hypotheses,

the formation of granite is essentially a mixing process. A granitizing agent (ichor, emanations, clouds of ions) penetrates and reacts with a heterogeneous mass of sediments and metamorphic rocks. The pre-existing rocks take just what they need from the invading medium and surrender to it exactly what they do not need. The end product is granite. It is practically impossible to discuss a process of this type in terms that are both general and meaningful, but we can come to grips with it if we are willing to consider variations exhibited by some constituent abundant both in the pre-existing rocks and in the end product.

Sandstone and quartzite, for instance, are a common part of any sedimentary or metamorphic complex. Schuchert[3] estimates that they form about 25 per cent of the sediments of the Appalachian trough, and we may use this as a first approximation for New England. (It makes little difference whether the correct value is 20 or 30 per cent.) From the work of Tallman,[4] we would anticipate that nearly half of this material carried over 95 per cent of quartz and that in about two thirds of it the quartz content was over 75 per cent. Of a little over 200 analyzed thin sections of New England granites in my collection, not one carries as much as 40 per cent of quartz, and in only three is the quartz content over 36 per cent. What has become of the sandstones and quartzites? Evidently they are gone without trace. Would we not anticipate that a feldspathization process so extreme would overshoot a little, forming, here and there, minute patches (of the order, say, of a few square inches or parts of a square foot) of syenite? It does not seem to, and any such patches which do occur must be extraordinarily rare. I have not so far found a single thin section of New England granite containing less than 20 per cent quartz. What kind of feldspathization process is it that works perfectly wherever it finds more than 40 per cent of quartz and fails completely when it reduces the quartz content to 20 per cent? Such a process operating on a very coarse scale and speaking only in terms of average or bulk compositions is hard enough to credit. What are we to say when it works at a scale of square inches of area and on volumes of the order of a thousandth of a cubic inch?

Unreasonable as all this seems, something of the sort must be involved in each of the nonmagmatic granitizations of highly siliceous sedimentary or metamorphic rocks. To the extent that this is so, I believe we must discard them outright as explanations of the origin of these particular granites. A similar argument could be made out for other minerals, particularly biotite, muscovite, and total feldspar, but it would necessarily be more tedious and less persuasive, since reliable quantitative information about the modal composition of metamorphic rocks is virtually lacking.

Relations between Quartz, Orthoclase, and Plagioclase. We have so far found strong reason for preferring hypotheses which presuppose the existence of a homogeneous parent material for each granite mass. The implicit assumption that this homogeneous parent must be magma is just the type of gratuitous extrapolation to which the anti-magmatists object so strenuously; and their objection is often quite proper. The extrapolation is always dictated by convenience, but in the present case there is much more to it than that. The reason for selecting a liquid as the homogeneous parent material is that in each granite so far examined, the relations between quartz, orthoclase, and plagioclase are just what would be expected in samples of a crystallizing liquid. In most of these cases, the relations actually developed would not be anticipated in samples of anything else.

In FIGURES 3 to 6, which show the proportions of quartz, orthoclase, and plagioclase found in thin sections of individual granite masses, a slanted rectangular field extends upwards from near the center of the base line to a little past the center of the triangle. This is the silica-rich end of the thermal valley in the system, nepheline-kaliophilite-silica. Its significance has been

admirably described by Dr. Bowen,[5] who showed that: (a) fractional crystallization of a liquid of basaltic composition would generate residual liquids lying in or near the thermal valley; and (b) in the vast majority of cases, compositions of individual glassy rocks or averages for rock glasses of different types do in fact lie in or very near the thermal valley.

Our argument has nothing to do with glassy rocks, average values, or fractional crystallization of basaltic liquid, yet the distribution of values for each individual granite mass shows a curiously systematic relation to the thermal valley.

For certain granites, as shown in FIGURE 3, *all* results lie in the valley, a phenomenon perhaps not so critical for eucrystalline rocks as for glasses. There are three examples of this type in my sample: Westerly, Rhode Island; Bradford, Rhode Island; and Milford, New Hampshire. In each of the other nine New England granites for which I have suitable data, some points lie outside the valley, but in only one (Westwood, Massachusetts) are they all outside. Thus, in eight of the nine cases there is some overlap of the valley boundaries, and the nature of this overlap is highly critical, for *in any particular granite, values lying outside the valley are all on the same side of the valley.**

In the granites of North Jay-Hallowell, Maine, Westwood, Massachusetts, and Pownal, Maine, outlying values are all on the orthoclase side of the valley (FIGURE 4 shows the data for Pownal). In the granites of Fitzwilliam and Concord, New Hampshire, and Clark Island, Maine, all overlap is in the quartz field (the data for Clark Island are shown in FIGURE 5). In the granites of North Sullivan, Maine, and Barre and Woodbury, Vermont, overlap is exclusively on the plagioclase border of the valley (FIGURE 6 shows the data for Woodbury).

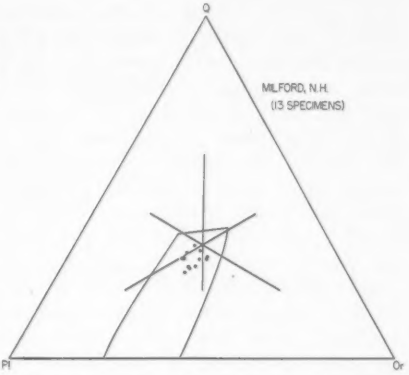

FIGURE 3. Proportions (by volume) of quartz (Q), plagioclase (Pl), and orthoclase (Or) in 13 thin sections of Milford, N. H. granite. All values in thermal valley.

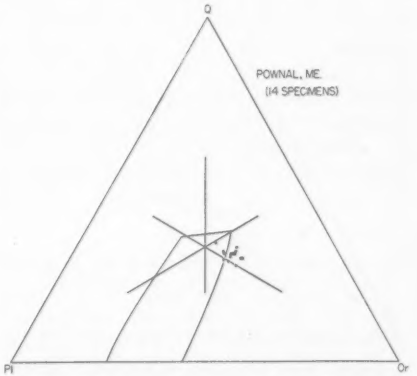

FIGURE 4. Fourteen thin sections of Pownal, Me., granite, showing overlap of thermal valley on orthoclase side. Coordinates as in FIGURE 3.

In every one of these nine cases, a reasonable shift of the ternary mean would have generated overlap on two sides of the valley. In three of the nine, dispersion is easily large enough that a rather small shift of the ternary mean, depending on its sense, would

* A few minor exceptions should perhaps be mentioned. Of a total of twenty-two thin sections of Barre, results for two lie not only well to the left of the plagioclase margin of the valley, but slightly above the boundary of the quartz field. This is true also for one of the eight slides of Woodbury and two of the ten slides of North Sullivan. In all four cases, the discrepancy is considerably smaller than analytical error.

It is to be noted, also, that the valley is outlined for the pure system Ab-Or-Q, while the modal values are for plagioclase containing some anorthite and orthoclase probably containing a little albite. It is hard to see how any such systematic relationship could develop unless, on the whole, the amount of Ab in Or was of the same order as the An content of plagioclase. The plagioclase of all these granites is normally intermediate oligoclase, albite and andesine both being extremely uncommon. The Ab content of modal orthoclase cannot be satisfactorily determined without extraction of the mineral from each rock. This work is now in progress.

have generated either a two- or threefold overlap. Yet the single overlap is the only type so far observed.

By any of the nonmagmatic hypotheses this effect must be regarded as a chance event. As far as these hypotheses are concerned, points might fall outside the valley on any one side, on any two sides, or on all three sides. Of these seven possibilities, only three are realized, and all are of one type; *viz.*, the single overlap. Because of the variability of dispersion, no exact probability can be attached to this event. A rather large scatter is required for a triple overlap, while the width of the valley clearly sets a maximum value for cases of no overlap. If we suppose, however, that single and double overlaps are equally likely, the probability of obtaining nine of one type and none of the other is only $(\frac{1}{2})^9$ or .002. If we suppose that single overlap is twice as likely as double, which is surely an overestimate, this probability is still only $(\frac{2}{3})^9$, or .026. The correct answer is probably somewhere between these limits. By standards usually applied in this type of work, the probability of the observed frequency of single overlaps is so small that its occurrence as a chance event is extremely unlikely. Hypotheses which can only regard it as a chance event (and this includes, I believe, *all* nonmagmatic hypotheses) are by the same token rendered extremely unlikely.

FIGURE 5. Thirteen thin sections of Clark Island, Me., granite, showing overlap of thermal valley on quartz side. Co-ordinates as in FIGURE 3.

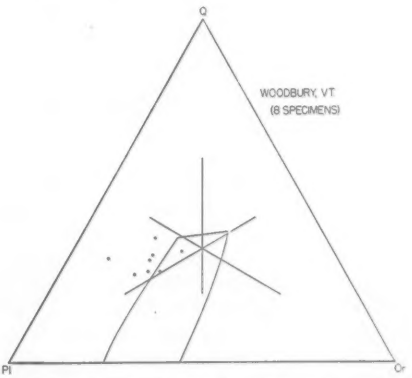

FIGURE 6. Eight thin sections of Woodbury, Vt., granite, showing overlap of thermal valley on plagioclase side. Co-ordinates as in FIGURE 3.

For magmatic hypotheses, the situation is entirely different. The observed distribution of Q-Or-Pl values is reasonable and almost necessary. We could tolerate occasional instances of double or triple overlap, of course, but if such cases proved numerous we would have to conclude either that the experimental technique was faulty or that the theory was wrong. Crystallization of a liquid whose initial composition lies in the diagram generates a liquid residue which approaches and finally enters the thermal valley, and, unless heat is added, the liquid cannot escape from the valley once it has entered. The composition of adequate samples of such a crystallizing mass would necessarily fall either in the valley or outside it on the side on which the initial composition lay.

Summary and Conclusions. The magmatic hypothesis (by which I mean simply the notion that each of these granite masses has formed by crystallization of a liquid of approximately the same composition as the finished product) thus survives two rather critical tests, both of which throw strong

doubt on the whole suite of nonmagmatic explanations. With regard to internal variations in composition (and quartz variation is only one of several possible examples), the nonmagmatic hypotheses would seem to require dispersion decidedly greater than that actually found. With regard to variations in the relative proportions of quartz, plagioclase, and orthoclase in each mass, they offer no reason for expecting any particular type of distribution and are unable to regard as more than a chance event the peculiarly systematic type of distribution which does actually emerge.

Now, it is not all smooth sailing for the magmatic hypotheses either. The conclusion that there is no *single* granite magma, first reached from the Q-M-F projections, is strongly affirmed by the Q-Pl-Or distributions. There are at least four distinguishable types of parent material, and it is not unreasonable to suppose that no two granite magmas are identical, even with regard to major constituents. Further, the whole discussion of Q-Pl-Or relations is something of a *tour-de-force*. The thermal valley is developed for the pure system Q-Or-Ab; in real granites there is invariably some anorthite in plagioclase and some albite in orthoclase. Although they complicate the discussion considerably, uncertainties introduced by this difficulty are probably rather small.

Finally, nothing has been said about the origin of magma, and in fact the evidence throws no new light on that problem. It is often argued that the magmatic origin of granites is suspect, because no known process could generate enough magma. But a pedestrian does not refuse to step out of the path of an approaching truck because he cannot be sure it was made in Detroit. I do not know how or where granite magmas get themselves manufactured, how they enter or move about in the crust, or how they manage to maintain their identity prior to crystallization. If, however, we seek a rational explanation of the variations in composition exhibited by the finer-grained calc-alkaline granites of New England, it seems to me we shall have to suppose that, in some fashion, they manage to do all three of these things.

The validity of this conclusion hinges, of course, on the adequacy of the sampling procedure, and the need for adequate sampling techniques in petrography is little short of desperate. The conclusion will no doubt be extremely unpopular in some quarters. It is useful to point out that merely questioning the sampling procedure is not enough. Anyone who dislikes the conclusion enough to want to do something about it will have to do a great deal. Briefly, he will have to develop a different sampling technique and obtain for samples collected in the new fashion a number of quantitative modal analyses at least comparable to that used here. Information accumulated in this fashion would be of infinitely greater value to the science than the groundless speculative quarrels which now occupy us. In the end, we would at least have something to talk about.

However this may be, I believe sufficient evidence has now been offered in support of the assertion that the availability of large numbers of reliable quantitative modal analyses would immediately and powerfully affect discussions of the origin of rocks. A systematic quantitative petrography would be well worth having.

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SECTION OF PHYSICS AND CHEMISTRY

THE SEARCH FOR LONG RANGE FORCES IN SOLUTION*

By BENJAMIN CARROLL†

In a series of publications,[1] Rothen has presented experimental evidence which strongly suggests that antigens and homologous antisera can react in the presence of an intervening film. The film, usually referred to as a blanket, may be several hundred Ångströms thick. This work has been extended to include enzymes. Here again it appears that direct contact between enzyme and substrate is not essential for enzymatic activity.

The Rothen technique is direct in its approach to this problem. A slide is covered with one or more monolayers of protein antigen. A film of known thickness of inert material, such as Formvar, polyvinyl chloride, or barium stearate, is deposited on top of the antigen film. Finally, a drop of dilute solution of the corresponding immune serum is spread over the blanket. After the slide is washed, specific adsorption of antibody can be detected. In the case of enzymes, a drop of enzyme solution is used in place of the immune serum. The effect of the enzyme acting through the blanket can be determined by testing subsequently for the specificity of the antigen film. Loss of specificity for homologous antisera indicates that enzymatic action has taken place across the inert blanket.

To account for this phenomenon, Rothen has postulated the existence of long range forces which can act over a distance of several hundred Ångströms.

Contrary to this viewpoint is the evidence that has accumulated from the work of many immunologists.[2] Their data indicate that serological specificity is dependent to a large degree upon "lock and key" complementariness as suggested originally by Paul Erlich. Pauling[3] and coworkers have shown that a portion of the antibody molecule fits the antigen group so well that immunochemical techniques may be used to determine the configuration of molecules and haptenic groups. They have shown that the forces involved in the specific combination of antibody and antigen require approximation of the attracting molecules to within one or two Ångströms.

To help resolve these conflicting viewpoints, we have looked for the Rothen effect in solution. Conditions similar to the Rothen experiment on enzymatic action were used. The substrate, bovine serum albumin, and the proteolytic enzyme, pepsin, were selected. The plan was to coat the albumin molecule with an inert layer or blanket by the adsorption of ions or molecules and then investigate the effect of the enzyme upon the coated substrate.

According to Rothen, long range forces should be exhibited if the bulk conductivity of the blanket is practically zero. In selecting substances to coat the albumin in solution the choice was limited more or less to paraffin chain salts or dyes. The use of paraffin chain salts was open to several objections. Their effect on proteins has been reported to be harsh, frequently resulting in the denaturation of the protein.[4] A more serious objection is the uncertainty in estimating the area on the substrate molecule that becomes inaccessible to the enzyme when a known number of paraffin chain molecules are adsorbed. Whether the paraffin chains would be closely adlineated, with their tails pointing toward the liquid phase, or randomly kinked or lying flat on the surface of the protein is unknown. In building up a blanket with dye molecules, the uncertainty in the estimated area of substrate blocked by a dye molecule is considerably less.

The number of dye molecules required to form a unimolecular blanket around the bovine albumin molecule, which has a molecular weight of 69,000, will depend upon the model taken for the protein as

* This paper, illustrated with lantern slides, was presented at the meeting of the Section, February 7, 1950.

† Newark Colleges of Rutgers University, Newark, N. J., and Department of Chemistry, Columbia University, New York, N. Y.

well as the dye. If the albumin molecule is assumed to be spherical, its surface area is about 10,000Å². Viscosity, dielectric dispersion, and diffusion data point to an asymmetric molecule.[5] These measurements are consistent with a model of a prolate ellipsoid having an axial ratio of 1:5. This would yield a surface area of some 25,000Å² for the albumin molecule.

Azo dyes having a molecular weight of about 350 were used for the blanket. The area of protein that would be covered by a dye molecule is 60Å² if the dye is considered cube-shaped and about 150Å² if it is spread flat on the surface of the protein. A convenient approximation of 100Å² taken as the average of these two extreme configurations indicates that 200 to 300 dye molecules spread evenly over the surface of a bovine serum albumin molecule may be considered a unimolecular blanket having a thickness of a few Ångströms.

At first, attempts were made to build up a blanket with so-called fat-soluble dyes.[6] Such a blanket was sought because the probability of having the enzyme rapidly displace the dye molecules and thus gain access to the substrate seemed remote. These attempts were abandoned because an insufficient number of dye molecules were adsorbed by the protein.

Water-soluble acid dyes which follow Beer's law were then used. Since the number of sites available to these dyes on the alkaline side of the isoelectric point of albumin[7] is about $\frac{1}{10}$ that required for a unimolecular layer, adsorption studies were undertaken in 0.01M HCl. The total concentration of dye was adjusted to a value that gave a reading in the sensitive range of the spectrophotometer, since this instrument was used to determine the amount of dye adsorbed by the protein. The extinction for 2.0×10^{-5} M Orange I in the presence of bovine albumin is shown in FIGURE 1.

The results are similar to those given by Klotz.[8] There are two points of difference however. One is that the extent of the adsorption is increased by several orders of magnitude. Secondly, the adsorption is distinctly a two-step process, where at first 100 to 130 dye molecules are adsorbed by a single albumin molecule. This occurs immediately upon mixing the dye and protein. In addition, there is a more deliberate uptake of dye over a period of several weeks, so that an apparent upper limit of some 250 dye molecules per protein mole-

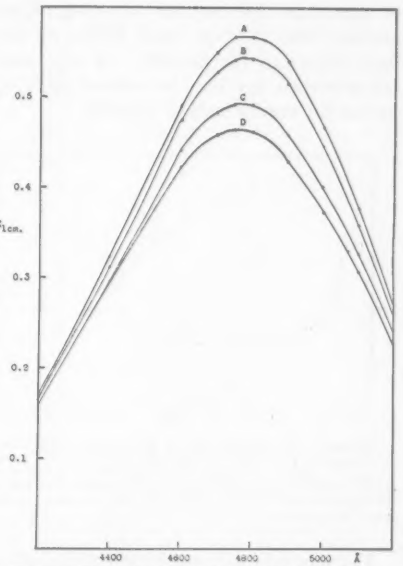

FIGURE 1. The extinction of 2.0×10^{-5}M Orange I in presence of crystallized bovine albumin and 0.01M HCl. Readings taken 10 minutes after mixing. Temperature 27°C. Concentration of bovine albumin in p.p.m.: A—0.0; B—2.0; C—10.0; D—100.0.

cule is attained. These results are indicated in FIGURE 2. It will be seen that in order to maintain this upper limit the molecular ratio of dye to albumin in the solution must be about 10^3. This means that, when one part per million of albumin is present in 2.0×10^{-5}M Orange I, a sufficient number of dye molecules is adsorbed to form a unimolecular blanket.

It was thought that the slow uptake of dye was due to the progressive denaturation of the albumin in 0.01M HCl. Experiments[9] indicated that bovine albumin was remarkably stable in acid solution and that

the coated albumin could be regenerated by dialysis. The binding capacity of the albumin for simple dye anions at pH 7 was used as a criterion of denaturation.[10]

A question to consider is the disposition of the dye molecules on the coated albumin. The fact that the bovine albumin molecule has 112 cationic groups would indicate that the initial adsorption of dye takes place at these sites.[11] The subsequent uptake of additional dye may be occurring either around these sites or, most likely, on the bare surface of the protein. In any case, the adsorbed dye may be viewed more or less as an evenly spread blanket.

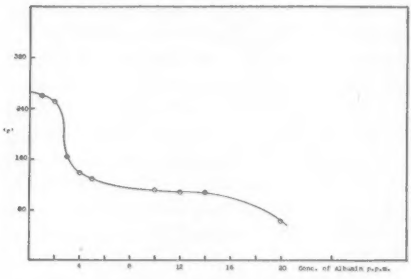

FIGURE 2. This graph shows (r) the number of dye molecules adsorbed per protein molecule after allowing 2.0×10^{-5}M Orange I to remain in contact with bovine albumin for 30 days at 0°C. Solutions were equilibrated for 6 hours at 27°C. before spectrophotometric readings were taken to determine the extent of adsorption.

The effect of a proteolytic enzyme on a substrate concentration as low as one part per million required the development of a new method of determining the activity of proteolytic enzymes.[12] The method had to detect any possible denaturing effect as well as hydrolysis of the substrate, since in the Rothen experiment either one or both effects may be responsible for the loss of specificity of an antigen.

It was observed that proteolytic enzymes caused the bovine albumin molecule to lose its combining capacity for simple dye anions. Spectral curves similar to those shown in FIGURE 1 were obtained for an albumin-pepsin solution when Orange I was present. As the reaction proceeded, the absorption curve approached, and in some instances reached, the spectral values for the dye alone. Since the products of hydrolysis of bovine albumin as well as of the enzyme had very little affinity for Orange I in 0.01M HCl, the changing spectral curves could be interpreted readily in terms of changing concentration of the native albumin. Such factors as temperature and initial concentration of substrate or enzyme were investigated, and the usual kinetic formulas could be applied.

The spectral method for detecting enzymatic activity was well suited to systems having initial concentration of substrate as small as one part per million. In addition, the method could detect the effects of one part per billion of pepsin. When a concentration of enzyme as large as 0.003 per cent was used, the half-life of the native albumin-pepsin reaction was always less than ten minutes and frequently less than one minute, the time depending upon the initial concentration of substrate.

To measure the velocity of the reaction, two procedures could be used. The dye could be present during the reaction or added to an aliquot of the dye-free reaction mixture at various time intervals. If 'r,' the molecular ratio of adsorbed dye to albumin, was small, both procedures gave the same results. As 'r' increased beyond the value of 100, the presence of the dye slowed down the reaction appreciably but did not stop it. This will be seen in FIGURE 3. The results in FIGURE 3 suggest that the loci of attachment of the dye at values of 'r' greater than 100 may be near the peptide linkages, because of the pronounced effect of the dye in inhibiting the reaction. As the value of 'r' increases, the reaction is slowed down to a point where, approximately, a unimolecular blanket inhibits the albumin-pepsin reaction completely. Similar results were obtained when the pepsin concentration was increased to 0.1 per cent. It is interesting to note that at 'r' = 114, where the albumin has its particle weight increased 50 per cent, the blanket offers little protection. Some may interpret this as a Rothen effect. Nevertheless, the fact that a blanket which

can be considered unimolecular in thickness completely inhibits the reaction between albumin and pepsin is strong evidence in favor of the viewpoint that intimate contact between substrate and enzyme is essential for enzymatic activity.

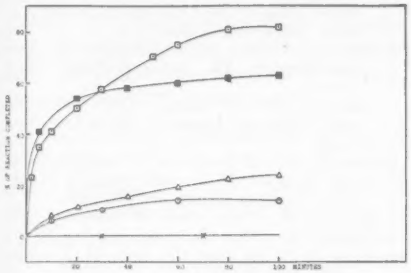

FIGURE 3. The effect of .003 per cent crystallized pepsin on albumin in presence of 2.0×10^{-6}M Orange I. Temperature 27° ± 0.1°C. Concentration of HCl is 0.01M.

Initial conc. of albumin p.p.m	*Initial ratio of adsorbed dye to albumin* 'r'
×××× 1	260
⊙⊙⊙⊙ 3	162
△△△△ 4	142
⊠⊠⊠⊠ 10	114
⊡⊡⊡⊡ 1000	1.4

The literature contains one type of experiment on the kinetics in solution of a protein-enzyme system which has been offered as possible evidence for the existence of the Rothen effect. It is the experiment of Northrup,[13] showing that the digestion of casein and of gelatin proceed at the same rate whether present in separate solutions or in the same solution. The enzyme used was trypsin. Apparently, the concentration of enzyme to substrate was sufficiently small to yield a reaction velocity of zero order.

The spectral method for enzymatic activity can measure the effect of enzyme on albumin in the presence of other substrates which do not adsorb the dye indicator. Orange I at 2.0×10^{-5}M is not adsorbed to any appreciable extent by gelatin in 0.01N HCl. The effect of gelatin on the rate of the albumin-pepsin reaction is shown in FIGURE 4. Initially, there is ten times as much gelatin as there is albumin, yet the velocity of the reaction is not appreciably affected. The reaction which is zero order has a half-life of 15 minutes. The final points on the curve represent the reaction at 90 per cent completion.

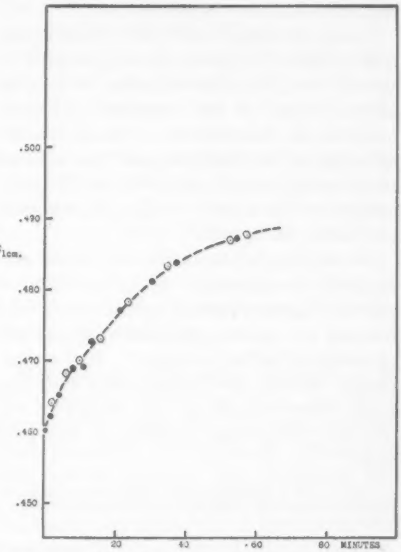

FIGURE 4. Effect of gelatin on reaction rate of crystallized bovine albumin (.016 per cent) and crystallized pepsin (4.0×10^{-6} per cent) in 0.01N HCl. Orange I (2.0×10^{-6}M) present during reaction; temperature, 30°C. The extinction of the reaction mixture at 4750Å is plotted as a function of time. ●●●●—reaction of albumin and pepsin in absence of gelatin; ⊙⊙⊙⊙—reaction of albumin and pepsin in presence of 0.16 per cent gelatin.

The results of this experiment are in complete accord with those of Northrup. The author is reluctant, however, to accept the postulate of long range forces as an explanation. Michaelis[14] has suggested that proteolytic enzymes may have enzymic sites with different specificity.

The implications of the Rothen effect in the natural sciences are obviously enormous. Unless the effect is demonstrated in the liquid and solid state, it is doubtful if the scientist will accept it. The reason for this is the practical and theoretical experience that has accumulated for these two

states of matter. By comparison, surface chemistry, particularly the behavior of films on solid surfaces, is a very recent field. Electron microscopy and electron diffraction are basic techniques in this subject but are relatively new and still inaccessible to most investigators. Undoubtedly, the Rothen effect will do much to stimulate research in surface chemistry.

It may be considered that special orientation effects in films normally absent in solution would be responsible for the Rothen effect. Some of the strongest evidence, however, for the existence of the intermediate complex in reactions and that contact between reactants is essential can be gathered from the kinetic studies of unimolecular films on liquids.[15]

In solution, hapten inhibition of antigen-antibody precipitation or the inhibition of protein-enzyme reactions by a unimolecular blanket are strong arguments against the existence of the Rothen effect. Perhaps the crucial test for the Rothen effect and long range forces would be the finding of appreciable enzymatic activity in the solid state.*

* The author is indebted to Prof. A. W. Thomas for his encouragement and helpful suggestions regarding this work.

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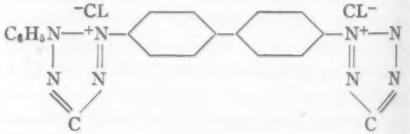
SECTION OF BIOLOGY

THE USE OF NEOTETRAZOLIUM AS A TOOL IN THE STUDY OF ACTIVE CELL PROCESSES*

By WILLIAM ANTOPOL, SUSI GLAUBACH, AND LESTER GOLDMAN†

The selective staining of tissue by pp′ diphenylene bis 2-(3,5 diphenyl tetrazolium chloride), *in vivo* and *in vitro*, was described in a previous report. The substance is called neotetrazolium and will hereafter be referred to as NT. The conversion of the pale yellow compound to an insoluble deep purple to black compound is due to the reduction of NT, forming a colored formazan which takes place at the site of active metabolic processes.[1] The formula for NT is



The reduction of NT is not reversible, and once the formazan is precipitated at the site of reduction in the living cell, it remains *in situ* for some time. It was therefore of

* This paper, illustrated with lantern slides, was the first of three papers presented at the meeting of the Section, February 13, 1950.

† The Joseph and Helen Yeamans Levy Foundation, Beth Israel Hospital, N. Y. and the Laboratories of the Newark Beth Israel Hospital, Newark, N. J.

interest to study the influence of chronic administration of NT in animals.

In this communication, we will report on the appearance of organs after intraperitoneal, subcutaneous, and oral administration of NT to white mice, the influence of intradermal injection of NT on the hair color of C_3H (brown), *dba* (gray), and C_{57} (black) mice and the effect of subcutaneous injection of NT on the growth of immature rats. In all the experiments, NT in a concentration of 0.1 per cent is used.

Intraperitoneal Administration of NT to White Mice. Sixty-nine male and female mice, weighing 25 to 30 grams, received daily injections of 0.5 cc. NT for periods varying from 1 to 60 days. The animals were sacrificed at varying intervals. In one hour, there was a slight pink tint with a purple hue to the peritoneum, and the omental and peritoneal fat stained faintly pink. At this time, there was a slight but distinct purple color in the liver and the cortex of the kidney. This was more pronounced after 24 hours. After 48 hours, the liver, kidney cortex, and outer areas of the medulla became deep purple. The mucosa of the stomach in the region of the gastric glands was faintly purple, while the nonglandular portion, the cardiac antrum, and the region containing the pyloric glands remained unaffected. In 3 days, the liver was deep purple to black. The kidney cortex also assumed this color and a faint color appeared in the medulla. The sublingual and submaxillary salivary glands and the mammary glands were purple; in the parotid, the color change was not as distinct. The intermediate lobe and, to a lesser degree, the anterior lobe of the pituitary, the interscapular fat pads, the bone marrow, and the prostate took on a purple tint. The reticularis zone of the adrenal cortex was distinctly purple. The ovaries, particularly the corpora lutea, also assumed this color. As time went on, the changes were more pronounced in all of these organs. The renal medulla became progressively darker, so that it approached the black appearance of the cortex. The entire animal took on a purple hue and most organs, including the lymph nodes, thymus, and muscle and lacrimal glands, were stained. The brain, testes, and seminal vesicles retained their natural color. The dorsal ganglia became darkly stained. The sympathetic ganglia were not seen.* Because of the normal purple color of the spleen, it was difficult to determine the degree of gross change in this organ.

When mice were treated on 4 successive days, after which treatment was discontinued, the staining of the abdominal organs was still intense 2 weeks after the last injection, becoming less distinct after 4 weeks. The kidney was pale; but a light purple color remained in the liver and the remaining intra-abdominal fat was still dark purple. When the mice were dosed continuously for 2 to 3 weeks, distinct staining of the organs could still be observed 3 months after the last injection.

Microscopic. Histologic examination of the tissues in which the color developed showed accumulation of formazan as fine black granules, at first in the perinuclear zone and, as time went on, throughout the cytoplasm. In the kidney, the NT reduction took place initially in a linear arrangement near the free end of the convoluted tubules. The localization and arrangement of the granules suggested that reduction of NT occurred in organoid structures of the cell cytoplasm. The color change occurred in the cytoplasm and was never observed in the nuclei of normal cells. Detailed histologic studies of each organ will be the subject of a future communication.

Subcutaneous Administration of NT to White Mice. In one series, twenty-five mice were injected subcutaneously with 0.5 cc. NT daily for from 1 to 5 days. In a second series, twenty-five mice received injections every other day for 2 months. Coloring of the organs took place as with the intraperitoneal administration, although

* The effect on the nervous system is being investigated in collaboration with Drs. Abner Wolf and David Cowen, College of Physicians and Surgeons, Columbia University, New York City.

at a slower rate. The intra-abdominal fat, however, remained unstained. If treatment was continued for longer than 3 weeks, the intra-abdominal fat diminished in amount or disappeared.

Oral Administration of NT to White Mice. Twenty white mice fed daily with 0.5 cc. to 1.0 cc. NT from 8 to 60 days showed color changes very irregularly which were considerably less pronounced than with subcutaneous injection. The liver and kidney changes were less irregular.

Administration of NT to Colored Mice. Twenty-two male and female brown mice (strain C_3H) were injected subcutaneously with 0.5 cc. NT every other day for 1 month and then mated. None of the females became pregnant, whether mated with NT-treated males or with untreated males. The appearance of the organs was similar to those in white mice. The ovaries were very small. The mammary glands also stained purple. Similar results were obtained in *dba* gray and C_{57} black mice. In a series of C_3H mice with spontaneous mammary tumors, the neoplasm stained intensely. Observations on the effect of NT on the occurrence of mammary tumors in this strain is under investigation, as well as on the effect of NT on the growth of mammary tumor *per se*.*

In the experiments with chronic subcutaneous administration of NT in C_3H mice, it was observed that in some mice bald areas and thickening of the skin appeared at the site of frequent NT injection. When hair grew back, it was not its natural brown color, but white. This phenomenon did not occur in the intraperitoneally treated mice. The question arose whether the appearance of white hair was not due to accidental intradermal injection of NT. To see the effect of intradermal injection of NT, the backs of male and female C_3H mice were partly shaved, and the next day the mice received a single intradermal injection of 0.5 cc. NT; the control mice received 0.5 cc. of NaCl (0.85 per cent). The hair in the control animals grew back in its natural color. In NT-injected mice it grew back white, and the white hair was only to be seen in the injected area. This experiment was repeated in *dba* gray and C_{57} black mice with the same results. The hair remained white until the mice died, the longest period of observation being 5 months. In one of the mice, the white hair was plucked out; the hair grew back white. It appears that NT injected intradermally, when reduced to its insoluble formazan might inactivate or interfere with the activity of enzyme systems concerned with pigmentation. Investigations in this direction are under study.

Subcutaneous Administration of NT to Immature White Rats. Fifteen young rats weighing 35 grams received subcutaneous injections of NT every other day starting with 0.3 cc. and increasing the dose to 0.5 cc. and then to 1.0 cc. TABLE 1 demonstrates a typical example of the growth-inhibiting effect of NT in these animals compared with control rats. It is worthy of note that the fur remains dull and immature in NT-treated animals even after 5 months. The color changes in the organs were similar to those observed in mice.

TABLE 1

	Control	*NT treated*	
Date	*body weight*	*body weight*	*cc. of 0.1 % NT every other day*
7–9–48	36 gm.	40 gm.	0.3
7–23–48	86 gm.	58 gm.	0.3
8–4–48	145 gm.	90 gm.	0.3
8–16–48	184 gm.	132 gm.	0.3
8–23–48	218 gm.	156 gm.	0.3
9–2–48	229 gm.	178 gm.	0.5
9–14–48	254 gm.	206 gm.	0.5
9–25–48	290 gm.	230 gm.	1.0
10–2–48	307 gm.	246 gm.	1.0
10–15–48	328 gm.	269 gm.	1.0
10–28–48	338 gm.	285 gm.	1.0
11–19–48	378 gm.	297 gm.	1.0

Discussion. The ability of NT to act as an hydrogen acceptor in the metabolic proc-

* The mammary tumor effects are under investigation in collaboration with Dr. Samuel Graff, College of Physicians and Surgeons, Columbia University, New York City.

esses of living cells and to be converted to the insoluble formazan at the site of reduction[1–3] makes it valuable not only as a tracer for intracellular localization of metabolic processes, but also as tool for determining whether certain drugs have a depressing effect on the activity of dehydrogenases. It is known that trivalent organic arsenical derivatives have a great affinity for —SH groups.[4] Since many dehydrogenases have free —SH groups which determine the activity of these enzymes, these derivatives have been used as inhibitors of dehydrogenases. We have shown that pretreatment of tissue *in vitro* with neoarsphenamine abolishes the ability of the tissue to reduce NT.[1] NT can also be used to demonstrate selective destruction or damage of certain structures in an organ (such as occurs in the kidney after administration of uranium salts to rats). Such a damaged kidney shows diminished ability to reduce NT *in vivo* and *in vitro*. This will be reported in detail in a subsequent communication.

NT is not only a tracer of hydrogen donors, it can also be an inhibitor of dehydrogenases. It has been observed that an *in vivo* injection of 0.5 cc. of a 0.1 per cent solution of NT into a rat muscle which is excised 24 hours later prevented this muscle from decolorizing methylene blue by the Thunberg method, in contrast to the corresponding untreated muscle of the opposite side.[1] These findings indicate that the process of reduction of NT and precipitation of formazan at the site of injection interferes with metabolic processes, *i.e.*, dehydrogenases. The findings herein reported (namely, the retarded growth and dullness of hair of immature rats treated subcutaneously with NT, the disappearance of intra-abdominal fat in NT-treated mice and rats, and the growth of white hair after a single intradermal injection of NT in gray, brown, and black mice) point again to disturbances in metabolic processes.

Of interest is the fact that spontaneous breast tumors reduce NT both *in vitro* and *in vivo*, while sarcoma 180 reduces NT very slightly *in vivo* but intensely *in vitro*. This poses the question whether NT lodges and is reduced in tumors arising from cells in organs which normally reduce NT *in vivo*. Investigations are in process to determine the effect of various tetrazolium and related compounds on tumors, particularly those which arise spontaneously in organs in which the tetrazolium compounds are reduced. The effect of compounds containing radioactive isotopes on these tumors will also be studied.*

It is also noteworthy that certain organs, such as brain and testes, and some tumors, such as sarcoma 180 and Flexner Jobling tumors, stain supravitally but not intravitally when injected with NT at a site distant from the tumor. The question of the existence of blood-tumor and blood-testis barriers should be considered as one does that of the blood-brain barrier.

The effects of NT and related compounds on viruses are of interest. We have already shown that certain organisms thrive in the presence of NT while others are retarded and still others are killed by it. The fact that certain viruses have active —SH groups and others do not indicates that the effects may very well differ with viruses as they do with bacteria.[1] Since certain tumors are caused by viruses, such as the milk-factor tumor of mice, the effect on the tumor as well as on the virus *per se* is to be studied.*

In studying the gross color changes, widespread as they are in chronically NT-treated animals, it becomes necessary to distinguish microscopically between the reduction of NT which takes place *in situ* and the mobilization and subsequent storage of colored compounds which are taken up by macrophages, and, to a lesser extent, endothelial cells, particularly in the liver and the adventitial zones of blood vessels. The different shades of color which can be seen in the organs raises the question whether they are due only to a variation of concentration of reduced NT, to different compounds, or to compounds in combination

* See footnote on p. 158.

with cell constituents. Extraction experiments with different organic solvents indicate that concentration alone cannot account for the various shades. For instance, ethyl alcohol at times extracts only part of the color, leaving a purple hue in the tissue, while acetone frequently extracts all the color.*

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* In addition to this paper, lantern slides were presented showing the *in vitro* reduction of NT in tumors, granulation tissue, corpus luteum of ovary, leucocytes in leukemic blood, and suspensions of tumor cells. The changes were striking under anaerobic conditions.

THE USE OF NEOTETRAZOLIUM IN THE STUDY OF *E. COLI* METABOLISM*

By HIROMICHI T. NARAHARA, HOWARD QUITTNER, LESTER GOLDMAN, AND WILLIAM ANTOPOL†

This paper is concerned with the use of neotetrazolium (NT)‡ as a tool for enzyme and cytochemical studies in bacteria. Work with *E. coli* is reported here.

Methods. The strain of *E. coli* used was isolated from a routine stool specimen. Plating the organism on Endo agar overnight produced uniformly red colonies. In our experiments, this strain was grown on enriched agar, washed, aerated, and suspended in Ringer phosphate solution buffered at pH 7.4. Experiments were set up in sterile, cotton-stoppered test tubes at 37° C. and conducted in duplicate or triplicate. 0.01 M concentrations of substrate and 1.3 mg. per cent of NT were present in the final 5.0 cc. volume of the mixture of organisms, substrate, NT, and buffer.

* This paper, illustrated with lantern slides, was the second of a series of three papers presented at the meeting of the Section, February 13, 1950.

† The Joseph and Helen Yeamans Levy Foundation, Beth Israel Hospital, N. Y. and the Laboratories of the Newark Beth Israel Hospital, Newark, N.J.

‡ pp′ diphenylene bis 2-(3, 5 diphenyl tetrazolium chloride). See preceding article.

Results. A. NT as an Indicator of Reduction Processes. The substrates alone did not reduce neotetrazolium. NT was not appreciably reduced aerobically by washed suspensions of *E. coli* but was reduced after the addition of solutions of the following compounds, which are known to be metabolized by the organism:[1–3] *sugars*—glucose, fructose, galactose, maltose, lactose, arabinose, and xylose; *polyhydric alcohols*—mannitol, sorbitol, and glycerol; *acids*—formate, lactate, pyruvate, succinate, fumarate, and acetate; *amino acids*—glutamate, asparagine, arginine, alanine, and glycine. Lag periods were noted in the utilization of lactose, xylose, and sorbitol.

Citrate and butyrate, which are poorly utilized by *E. coli*, produced at most a trace of formazan. Dead bacteria plus substrate did not reduce NT, indicating that active metabolism is required to produce a fair degree of NT reduction. Experiments conducted anaerobically proceeded at a faster rate than the same experiments done aerobically. It must be stressed that washed suspensions anaerobically showed good reduction of NT without added substrate, in contrast to the absence of reduction by similar suspensions aerobically. This is analagous to the increased reduction already described for tissues under anaerobic conditions.[4, 5]

B. Adaptation to Substrates. The lag periods in NT reduction by lactose and sorbitol were studied. Reduction of NT began after a lapse of 1–4 hours with lactose and of about 6 hours with sorbitol. Once reduction had started, it proceeded at a fairly rapid rate. Wet preparations made when gross color had just appeared in the tubes revealed formazan granules in practically every cell.[4] No granules were discernible in specimens examined prior to the development of gross color. Growth of the bacteria in media containing the specific substrate before preparing the suspension eliminated the lag period. The incubation of the buffered substrates for 34 hours before the addition of the bacterial suspension

also eliminated the lag period for sorbitol but not for lactose. The delayed reduction of NT corresponds to the absence of lactose fermentation, and apparently this phenomenon represents the period of bacterial adaptation. In the case of sorbitol, however, either adaptation or a change in the substrate by pH and long preincubation may account for absence of the lag period.

C. Morphology. Formazan was seen in the cells as discrete granules without diffuse staining. In all cells and with each substrate used, these granules were seen in a subpolar location characteristic for this strain of *E. coli* and for several other strains examined but not reported here. Occasionally, smaller cells had a single granule near one end. Most cells, however, possessed one granule in each polar region, and the larger cells often had a third eccentric deposit in the midportion of the cell. As our experiments progressed, the granules slowly grew in size at approximately the same rate in all cells. In *E. coli* K-12, Lederberg,[6] using triphenyltetrazolium, observed a single large formazan granule near one pole of the cell.

Attempts to stain killed bacteria with formazan in alcohol solution or by reducing NT chemically to formazan in the presence of dead bacteria were unsuccessful. This indicates that the sites of formazan localization are the sites of NT reduction by various dehydrogenase systems. Our studies suggest the presence of organoid polar areas of dehydrogenase activity in *E. coli*. These areas may be analogous to cytoplasmic organoid structures of plant and animal cells.

Summary. (1) A large variety of organic substrates known to be utilized by the organism have been shown to reduce neotetrazolium to its colored, insoluble formazan in the presence of washed suspensions of actively metabolizing *E. coli*.

(2) Direct examination of bacteria exposed to NT and substrate allows for the detection and rough estimation of the enzyme activity of the individual cell.

(3) The lag period in lactose and sorbitol utilization is characterized by the absence of NT reduction.

(4) The regular appearance of formazan granules in the polar regions of the bacterial cell suggest that these areas are organoid and represent the sites of dehydrogenases.

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INFLUENCE OF TETRAZOLIUM SALT ON THE GROWTH AND CYTOLOGY OF ROOT TIPS*

By B. P. SONNENBLICK, W. ANTOPOL, AND L. GOLDMAN†

Neotetrazolium, a new tetrazolium derivative, has been utilized in a series of investigations concerned with root tip growth and cytology. This redox indicator, slightly yellow in color, has several advantages over tetrazolium salts previously used in studies of cellular physiology, as indicated by Antopol, Glaubach, and Goldman.[1] The stock solution of neotetrazolium (abbreviated as NT) was 0.5 per cent in strength. NT in 0.85 per cent sodium chloride gave similar results to those employing NT in tap water. The latter solution was then used, since it could be applied directly to the jars containing the growing roots. Concentrations of NT ranging from stock strength to 1 part NT in 2 million parts water were used, and *in vivo* as well as supravital (excised roots) effects

* This paper, illustrated with lantern slides, was the third of a series of three papers presented at the meeting of the Section, February 13, 1950.

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were studied. Root tips of the onion, *Allium cepa L.*, were primarily employed in our tests.

The most striking gross effect was the progressive color changes in the root, particularly in the active mitotic zone above the root cap. The tinctorial changes ranged from delicate pink through red to a stable purple-black coloration. The appearance of color was closely observed and it was noted that some areas within the zone showed color earlier and in greater intensity than other areas, presenting a mottled appearance. Shortly, depending on NT concentration and time of exposure, the entire meristem darkened, whereas the older differentiating portion of the root appeared unchanged or acquired a pinkish tint. A similar but not so marked effect was noted in the primary root of radish seedlings. The reduction of relatively colorless tetrazolium salts to yield colored formazan compounds and the accumulation of the latter within the embryonic plant cells result in the macroscopic changes mentioned. No gross color changes were seen after exposure to 2,3 diphenyl 5 methyl tetrazolium chloride in 0.5 per cent concentration. Reduction of 2,3,5 triphenyl tetrazolium chloride, however, can occur in excised root tips; but the effect is not so specific as with NT. With the former, the entire root is colored pink to red, and the meristem more intensely, and the red formazan begins to diffuse out of the cells by 1–2 hours after the start of a test, tinting the medium. Results with neotetrazolium are obviously not similar to those where studies with other tetrazolium derivatives have been used.

Tests were made to determine the influence on the growth of young roots of chronic exposure to NT in concentrations ranging from 11 ppm. to 0.5 ppm. (1 part NT in 2 million parts of water). With the strongest NT concentrations, color appeared earlier, but within 3–6 days such roots were behind in growth as compared with controls and also with roots given weaker NT treatments. Many such tips were abnormal in pigmentation and gross morphology. In root tips exposed to 0.5 ppm., color was visible only after 13–18 days of exposure as a diffuse but delicate pinkish tint. Growth of these tips was approximately that of the controls. In one experiment, root tips given this treatment showed better growth than those of two of four control bulbs. Dosages of from 1 part NT in 7,500 parts water to 1 in 12,000 parts (with 6–8 hours exposure) were in the sublethal zone of effect, since some of the roots, but not all, evinced subsequent growth. Tips exposed to concentrations stronger than these for the same time became flaccid and did not grow. Treatments which were lethal to developing young roots did not prevent the appearance and growth of additional roots from the same bulb, nor did such toxic root treatments influence growth of the shoots.

In sections of treated root tips, it is possible to study the orientation and appearance of the colored formazan compounds which eventuate after reduction of the tetrazolium salt. Ordinary dehydration and clearing procedures resulted in the loss of most of the dark granules, and recourse had to be made to frozen sections. It became possible, however, to demonstrate black formazan granules in thin paraffinized sections, and the method has been employed with diverse plant and animal tissues. Fixation in neutral 10 per cent formalin and dehydration in tertiary butyl alcohol is followed by several changes of petrolic ether, by a 1:1 petrolic ether-paraffin mixture, and then by embedding in paraffin. After sectioning, no cytoplasmic stain is used; the sections are either left unstained or stained only with weak hematoxylin.

The reduced substance appears within the cells as solitary black granules or aggregates of granules grouped characteristically about the nuclear membrane. While this perinuclear orientation of the formazan granules is marked in the cytoplasm, it would appear that there is no intranuclear deposit of similar granules. Following triphenyl tetrazolium treatment, stellate crystals or crystal fragments may be seen in the cytoplasm, and occasionally such crystals are located about the nuclear membrane.

The effects of neotetrazolium on nuclei were studied and some tentative comments may be made. Treatments were given in six graded concentrations (from 1:5,000 to 1:500,000). Tips were removed and fixed at successive 2-hour intervals for 24 hours, and then at 36 and 48 hours. There is no colchicine-like accumulation of metaphase figures with abnormally contracted chromosomes. With higher concentrations and increasing exposure, there is disruption of cell structure, with enhanced vacuolation and shredding of the cytoplasm to form thin, irregular strands, while after weaker treatment cytoplasmic organization persists. The nuclear effect of higher concentrations is a progressive diminution in the number of mitotic figures, the effect varying with dosage and length of treatment. The eventual picture may be either a mass of pycnotic nuclei of irregular contour or of pycnotic interphase nuclei plus an occasional prophase. At the lowest concentration used, the mitotic figure, with respect to spindle and chromosomal structure as well as the frequency of the different stages, was essentially unaltered. There is some evidence, however, that low concentrations may in fact stimulate mitoses to exceed those in control material. The influence of NT appears to be primarily cytoplasmic, and the gradual cellular alterations are probably results of interference with cellular respiratory processes.[1, 2] From the typical perinuclear concentration of reduced tetrazolium products, there may also follow modifications in cellular protein syntheses. Smear preparations of root tips show formazan deposits around the walls of the differentiating cells. Pratt and Dufrenoy[3] have reported that triphenyl tetrazolium reduction can occur about the pores of sieve tubes and other intercellular connections in the stalk of sugar cane.

The influence of temperature on the reactions leading to NT reduction within plant cells was studied. The roots were treated *in vivo*. Then they were cut and placed in stock NT solution and the reaction was timed. Time and intensity (the latter arbitrarily quantitated in degrees from zero to plus four) were recorded by two observers. Treatment at 70°C. for as little as 15 seconds resulted in no reduction of the salt. At 65°C., after 15 seconds of treatment, color intensity of the meristem of exposed root tips exceeded that of controls for approximately 20 minutes. After that they fell behind, while the controls progressed in intensity to an end point of plus four. The treated roots did not reach that end point. After 30 seconds of treatment (65°C.), the experimental tips were behind the controls in speed and intensity of the reaction from the beginning, while 90 seconds of exposure gave no subsequent color reaction. At 60°C., exposures for 15 and 30 seconds gave the experimental tips a lead in the reaction for one-half hour, after which controls and experimental material showed approximately similar responses and proceeded to a similar end point. Studies at the aforementioned temperatures, and others, lead to the conclusion that 60°C. is optimal for the reaction in onion root tips.

Tests at low temperatures have been performed (from 2°C.–10°C., and for periods ranging from 1 hour to 8 days), and it has been found that after such exposures the reaction, as determined by color intensity, is speeded up above that of the controls. The enzyme or enzymes involved in the reaction are not inhibited at the lower temperatures and speed and intensity are greater than in untreated roots. Although it was slight, tetrazolium reduction occurred even in excised roots which had been kept frozen for 30 minutes on the frozen section machine. These data permit some varied speculations.

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SECTION OF PSYCHOLOGY

AN INTRODUCTION TO THE WORK ON EXTRASENSORY PERCEPTION*

By J. B. RHINE†

We can all agree that in our search for a fuller understanding of man's nature we must overlook no source of relevant facts. We can agree, too, that the more challenging a claim may be, the more significant it is likely to be if it can be sufficiently well established.

This is all the agreement we need to take up the case for extrasensory perception or ESP (the ability to acquire knowledge of, or respond to, an external object without the use of the senses). As with any research, we must look to our methods and draw conclusions only from results that allow no alternative explanation.

For most, if not indeed for all, of those who are familiar with the great accumulation of results of ESP researches, the occurrence of ESP is established fact. Accordingly, in drawing the attention of a group still unacquainted with the field to some of the evidence of ESP, we need only to turn to records long since published, not to new or even recent experimentation. I can, in fact, think of no better methodological exhibit with which to begin than the experiment at the Duke Parapsychology Laboratory on which we based the first published conclusions that we had evidence of extrasensory perception.

This experiment, known as the "Pearce-Pratt Series," which was published in the present author's first monograph,[10] consisted of three hundred trials of card-calling tests, or 12 runs of 25 trials each. Dr. J. G. Pratt, then a graduate assistant in psychology, conducted the experiment, with a Divinity School student, Hubert Pearce, as the subject. In one building, Pratt would place the ESP-test cards face down in the center of the table in front of him at a rate of one per minute and without looking at them. In another building, 100 yards distant, Pearce would attempt to identify them as they were being laid down. Duplicate records were made and turned over to me before the two men met after the test.

The total number of hits for the 12 runs was 119. The number to be expected by chance is only 60, or five per run. The probability is less than one in a million billion that a deviation this large or larger would occur by chance alone in such a series. Such was the crucial test of ESP which led us to decide to publish, in 1934, a full report of the first three years' research. After that report, the Pearce-Pratt series was continued with a comparable scoring rate for 62 more runs, some at a greater distance and some with myself as witness. Any question concerning the data must be focused on the method used; and no essential weakness therein has so far been called to our attention.

In 1939, a group of seven experimental psychologists[1] described a set of conditions which, in their judgment, would have to be used in crucially testing for extrasensory perception. It was decided at the Duke Laboratory to meet this challenge with a special research, and for good measure a number of additional safeguards were added. This resulted in what is known as the Pratt-Woodruff research series.[9] In this work, the two experimenters, Drs. Pratt and J. L. Woodruff (who was then a graduate assistant in the Laboratory), used as subjects 32 volunteers, mostly students at Duke University, selected on the basis of interest in being tested.

The conditions of this experiment are far too elaborate for any complete description here. They require five pages of fine print in the published report, and were of such a nature that no error, inadvertent or otherwise, could be made by anyone without its being detected and either corrected or rendered ineffective in the evaluation of

* This paper was presented at the meeting of the Section, February 20, 1950.

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the results. The degree of independence in observing, checking, and evaluating results and the amount of safeguarding with locked boxes, officially stamped and numbered record sheets, and duplicate recordings surpass that of any other psychological experiment with which I am acquanited. Furthermore, the results of this elaborate experiment were definitely significant, giving a critical ratio of five. Again, no one has ever suggested any explanation but extrasensory perception.

Until and unless there is some legitimate question raised regarding the quality of these experiments, there is no great point to multiplying them and certainly no need to list further samples here. I might, however, mention some further types of evidence. There are many varieties of this kind of evidence in the ESP literature.

As soon as time and attention were given to the matter, many interesting observations were made regarding the distribution of hits in ESP tests. It was found, for instance, that in certain types of tests there was a tendency to score best at the beginning of a run and to decline to a chance level half-way through the run. If the subject knew when he was approaching the end of the run, he was likely to improve and thus produce a U-shaped curve for the run as a whole (something like the memory curve). The study of these position effects led to the discovery of many similarities between the hit distributions of different researches. The most interesting of these position effects is that in which the five segments of the run (five trials in each segment) show a curve of distribution somewhat like that of the run as a whole. The composite curve of these segmental "curvelets" showed a significant covariation with the curve of the run itself.[12] Dr. B. M. Humphrey and the writer pursued this position-effect study through a number of series,[7, 13, 14] finding significant covariation present even in series in which the overall average score was not itself significant. Such wholly independent evidence of unanticipated lawfulness in the ESP data transcends the ordinary issues that have been raised by the research and leaves no alternative to the ESP hypothesis.

Still later came a somewhat overshadowing position effect of a different type, first given wide attention in the work of Carington[2] in England. I refer to displacement, or the tendency of the subject to scatter his fire, as it were, from the main target or stimulus object to the adjacent ones. Following Carington's suggestion, Dr. S. B. Soal[18] examined his own ESP records and found highly significant evidence of displacement, both to the next preceding and to the next following target cards.

Carrying this matter of displacement or "near missing" one step further brings out a rather clinching order of evidence, still a subject of current and hence unpublished research. Last year at the Duke Laboratory, Cadoret found that, in certain series of records reported earlier,[3] certain subjects tended consistently to mistake one symbol for another (*e.g.*, the circle might be regularly mistaken for the square or the cross for the star). Dr. Pratt examined the Martin and Stribic[3] series of card-calling ESP data produced by one subject and found that in the 1800 runs so far analyzed the subject not only displaced to neighboring targets to a remarkably high degree but appeared also to be consistent in mistaking one symbol for another. This analysis shows what we might call displacement both to the wrong position and to the wrong symbol, a two-way "scattering of fire."

So much for the experimental aspect of the ESP research. Little space will be used here to discuss the mathematical methods, for they are standard procedures. We have been fortunate in having the close co-operation and guidance of some of the ablest of the mathematicians of probability. For a time, Drs. Joseph A. Greenwood and T. N. E. Greville have both served as statistical editors of *The Journal of Parapsychology*. At an early stage of the ESP controversy, criticism by nonmathematicians drew from the Institute of Mathe-

matical Statistics a statement of general endorsement of the methods of evaluation in use. Thus far, no one has found the research methods in error to an extent that altered the conclusions drawn in any case.

We have, of course, been taking ESP as an established reality for over fifteen years. In that time, some progress has been made beyond the mere conclusion itself, though by no means anything like what could have been achieved had we been able to go on with the research without fighting a long battle over the question of the occurrence of ESP, or if there had been accessible to us the financial resources for research that are available to any recognized branch of psychology. These limitations should be kept in mind in considering some of the progress made toward understanding the nature of ESP.

First of all, we find that ESP is very much an unconscious operation. The subject has no awareness as to how he experiences the symbol on the card or what happens before he decides whether to put down a star or a circle. Introspection provides him with no reliable information. He may have his own special way of selecting the symbol. He may hallucinate the symbol on the wall, he may take the first image that comes into consciousness, he may have a verbal impression, or he may have no idea as to how he is making his choice.

It is a significant fact that, when Dr. B. F. Riess's[15, 16] subject scored over 18 hits per 25 over a 74-run series and later, after an illness, scored at only the chance average of five, she had no idea that she was doing any differently in one case than in the other. Here is the main source of the difficulty, not only in the research on ESP, but in making any practical use of the capacity. So long as the subject is unaware as to how his ability works and when his impression is received, he can have no reliable conviction as to whether he is right. This unconsciousness naturally is a great barrier to the development of the ability. It is difficult to improve without awareness of success. In a few exceptional cases and in certain spontaneous experiences, however, it is clear that certainty is possible. We do not yet know the necessary conditions, but the discovery of them is now one of the main goals of the research.

We can appreciate the handicap of this unconscious nature of ESP better when we realize that it carries with it not only the uncertainty as to rightness and wrongness, but that it is equally likely to lead the subject to avoid the target (*i.e.*, unconsciously but systematically to miss it or reject it). This might be due either to unconscious negativism or merely to wrong method in making responses. Any consistent following of a mistaken method would lead to a negative deviation from mean chance expectation. A subject might, in the second half of his test session, go into some kind of a perverse mood, or shift his tactics without realizing he was doing it, thereby getting negative, not just chance, results and wiping out all the positive results given during the first half. It is obvious, then, that we have this "sign factor" to deal with in an ESP test, as well as the amount of the ability itself. Two subjects with equal ESP ability, one with a somewhat positive turn and the other with a negative twist, would cancel each other out if they did the same number of runs.

Dr. Gertrude Schmeidler, of the College of the City of New York, has found repeatedly[17] that, among her subjects, those classified as "sheep" (the ones willing to accept the idea of ESP) give ESP scores averaging above mean chance expectation, while subjects designated as "goats" (those who reject the possibility of ESP) average below the expected mean. This separation has repeatedly given significant differences. When Dr. Schmeidler, using Rorschach responses as evaluated by the Monroe Inspection Method, makes a further breakdown of her sheep and goat subjects who have taken the ESP tests, she finds that her highest scoring sheep are those that are both well-adjusted and nonconstricted,

while goats with these same characteristics give the largest *negative* deviation. This Rorschach separation looks more like one dealing directly with the amount of ESP ability itself; but it is not necessarily so. It is likely that a number of complex personality operations are involved in ESP, and all these would naturally have their own conditions.

Another of these methods of separating high and low scorers was discovered by Dr. Humphrey,[4] of the Parapsychology Laboratory at Duke, in her use of Elkisch's diagnostic technique based on the form quality of a subject's drawings. Dr. Humphrey found in some preliminary tests that the "expansiveness" or "compressiveness" of the subject's drawing was associated with his ESP score, and she concentrated upon this criterion alone. On the basis of these ratings of drawings, she has now had repeated success in separating high and low scorers when the ESP test itself was based upon a drawing of a concealed target picture. To some extent, the same trend has been found when the ESP test was based entirely upon the card-test method with the drawing only accessory. Dr. Humphrey's method, like the sheep-goat classification, separates the subjects who score above expectation from those who score below. In the simple card-calling test, the compressives tend to give a negative deviation and the expansives a positive one. The amount of ESP ability, however, may very well be the same, and "sign" the only variant.

The late Dr. C. E. Stuart,[19, 5] also of the Duke Laboratory, introduced an interest test which was given to the subject for response as to likes and dislikes. On the basis of the subjects' responses, Stuart worked out a scale of rating in which those expressing the greatest number of strong likes or dislikes were called "extremes," and those who were more moderate were called the "midrange" group. By dividing the subjects into midrange and extreme groups, he found that on a criterion series he could separate those giving positive deviations from those giving negative deviations. Applying the method to sixteen new series, with the help of Dr. Humphrey, he produced a significant separation of the high- and low-scoring subjects in these series taken as a whole. More recently,[6] Dr. Humphrey has extended the discrimination value of the Stuart Interest Inventory by the development of a 14-item scale, which gives a still better division of the subjects of the same sixteen series into high and low scorers and on an independent principle of separation.

The combination of the expansion-compression criterion and the two interest criteria, which are independent of each other, gives a separation that is better in terms of average scores than that of any two of the three methods used. Naturally, however, as the research advances, we hope to move much closer to the point where we will be able to say what kind of states and traits of personality are associated with high or low scoring in the ESP tests. The construction of special tests for the selection of the personality best fitted for good performance in ESP tests is under way.

It is not possible to review here the studies made on ESP to find its relation to space and time. These studies have, for the most part, already been published or soon will be; and a fairly up-to-date review may be had from the book, *The Reach of The Mind*.[11] These tests of the relation of certain mental processes to the external physical environment are, in my judgment, by far the most important of the researches in parapsychology. They cannot, however, be fully appreciated in brief review. Moreover, they are extremely revolutionary to the current materialistic philosophy which prevails in the sciences. Accordingly, these experiments on ESP in time and space may well be left out here, and reference made to them (in the pages of the *Journal of Parapsychology*) for those readers whose interest is sufficient for a further, and indeed larger, step outside the restrictive lines of conservative psychological doctrine.

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DIVISON OF MYCOLOGY

ISOLATION OF CULTURABLE FUNGI FROM DIVERSE HABITATS*

By B. M. DUGGAR†

In the sense here employed, the "isolation" of a fungus has reference to the preparation of that type of culture now generally known as a pure culture; that is, to the establishment and active growth in otherwise sterile, nutrient media of a single strain or species of fungus, free from any other living organism. The presence of any other organism would constitute a "contamination." The term "isolate" may be applied to any form or species thus segregated. The general purpose of isolation is, of course, to study or utilize this particular organism uninfluenced by any other biotic effects. In the case of many culturable fungi, such cultures as those just referred to are of first importance in order to proceed with any intensive studies. I shall try to be more specific by enumerating a few examples of this need.

As a likely procedure in tracing accurately the structural and developmental characteristics—the life history—of the organism, such cultures may be essential and will always be helpful.

A pure culture is a requisite if the fungus is to be studied in respect to its nutritional requirements or in determining the significant relations and responses of the organism to temperature, light, or other environmental factors. The plant pathologist, and indeed the human pathologist and dermatologist, may find it important for diagnostic purposes and, of course, a source of inoculum both for tests of pathogenicity, when these are desirable, and for *in vitro* tests in relation to fungicides and therapeutic agents.

Usually, the pure culture is indispensable

* This paper, illustrated with lantern slides, was presented at the meeting of the Division, February 24, 1950.

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in fermentative work in which various products of metabolism are collected and chemically isolated, as in the production of antibiotics. In certain brewing processes and dairy-product manufactures, however, the pure culture may be essential only as a starter, the fermentation being carried out otherwise with less consideration for sterile materials. Obviously, the metabolic products of the fermentation may soon limit contamination.

Respecting high lights in the development of biological isolation as a standardized procedure, I shall limit the discussion drastically. Directly upon the heels of improvements in heat sterilization of media and utensils, during the latter part of the past century, the problems of isolation appeared in the pathway of both bacteriologists and mycologists. As early as 1873, Klebs described a "fractional" procedure. About five years later, Lister laid the foundation, I would say, for the "dilution" method, which, in one form or another, constitutes our major technique of today. The earlier applications of the method were burdensome, but not impossible. Essentially, the program was this: material containing, or supposed to contain, cells or spores of the organism was diluted with sterile water to such an extent that a drop of the final suspension would contain, on the average (and preferably) not more than a single cell. It may be inferred that the Lister procedure, if accurately carried out, would be tedious in the extreme. In further development, the higher dilutions were also spread lightly over the surface of a sterile slice or block of potato, so that with growth the colonies were often widely separated one from another. Thus, solid media came into the picture.

About 1881, Koch's leveled plate technique was introduced, the medium being first gelatin and later agar. That was the beginning of a new era. At this point, one should be reminded, however, that, with gelatin and agar available as solid media, the Esmarck rolled tube appeared as a modification of the Koch procedure. Six years after Koch's paper, Petri suggested a further variation from the plate procedure, using instead the covered dish which is now a central item in almost any isolation program.

In order to bring my remarks regarding current usage in isolation procedures within the limits of time and space available, and probably more in line with current interest, I shall omit any consideration of isolation as applied to anaerobic or marine conditions or to many other specialized types of environment. The following outline will suggest some of the procedures requiring brief attention:

A. The dilution series and some modifications.
   (1) The routine dilution series with nutrient agar.
   (2) Dilution with elimination of the heat-sensitive.
   (3) Dilution with change of pH of the agar.
   (4) Dilution with elimination of the antibiotic-sensitive.
   (5) Dilution with other agents differentially deleterious.
   (6) Dilution with dye indicators.
   (7) Dilution after "enrichment" of the substrate.
   (8) Dilution with changes in the nutrient agar.
   (9) Dilution with flooding.

B. Special sources of spores or of other growth-competent cells or tissues.
   (1) Promotion of spore formation.
   (2) Exudation of gelatinous spore masses.
   (3) Spore discharge from sporangia, perithecia, apothecia, *etc.*
   (4) Special cases in plant and animal pathology.

C. The direct transfer method.
   (1) The case of the fleshy fungi.
   (2) The case of rapidly invaded host tissues.

D. The single-cell isolation (not here considered).

E. Special procedures with the water molds or aquatic fungi.

In practice, the routine dilution series with nutrient agar may be greatly modified by the mycologist, plant pathologist, dermatologist, or bacteriologist, depending upon, among other considerations, (1) the nature of the materials from which isolations are to be made, (2) the general characteristics of the organisms sought or expected, and (3) the population density and diversity. Assuming that the material consists of samples of garden, agricultural, forest-land, and grass-land soils and that the objective is the isolation of common molds and Actinomycetes, no great departure need be made from the routine performance except as it may be expedient to attempt, at the same time, the elimination of certain heat-sensitive organisms, especially bacteria. In this case, the procedure would involve two parallel dilution series, one with and one without the application of heat.

With adequate soil samples, 4–5 grams of soil are placed in a test tube and this is shaken vigorously after adding 15–20 ml. of distilled water. Allowing 10 minutes for sedimentation, and using a sterile pipette, about 10 drops from the midsection of the supernatant are transferred to a tube of sterile physiological salt solution (or sterile tap water). This constitutes tube 1, from which eventually about 5 drops are transferred to plate 1 of the 3-plate dilution series. From tube 1, at any dilution desired, tube 2 is prepared, and so on for tube 3, each of these tubes serving as inoculum for the corresponding Petri plate. As soon as the tubes have furnished inocula for the regular series, and if no duplicates were arranged, they are heat-treated, commonly at 53° C. for 15 minutes. In general, incubation is at 28°C., and 4–6 days may be required before transfer to agar of Streptomyces colonies is practicable.

In order to promote diversity among the colonies appearing on the isolation plates, the agar for the four plates may be adjusted to any pH desired within favorable limits for any species sought. Parallel series may be arranged at different pH values.

The development of antibiotics has made possible the use of these antimicrobial substances, at least those with strongly differential potencies, to exclude certain classes of bacteria from the plates to the advantage of certain molds, yeasts, and Actinomycetes. Aureomycin at a concentration of 0.25γ or more per ml. has been notably helpful. Penicillin is useful where the flora is made up of many Gram-positive spore formers. In such situations, the antibiotic is a new tool.

Other toxic substances may be serviceable in special cases. Norris has shown that common species of *Chaetomium* have a tolerance for calcium hypochlorite so high that the use of this agent greatly simplifies isolation. Dye materials and certain substances displaying visible oxidation or reduction changes have been used in bacteriology chiefly for indicator effects, but some dyes may be serviceable also in differential isolation.

The preparation of dilution cultures after enrichment of the substrate (for example, of the soil), by the addition of special nutrients known to promote the growth of organisms desired, has been practiced to advantage in soil bacteriology, but it appears that enrichment has been used by mycologists chiefly through the use of highly complex decoctions or infusions, as in the use of manure decoction to promote isolation of coprophilous fungi. Obviously, any important modification of the nutrient agar, arranged to be used in comparison tests with the standard agar, might be selectively serviceable.

In the several preceding paragraphs it is assumed that the isolation inoculum is eventually diffused in the agar of the plate. It is always possible, however, in each case separately, to flood the plate with the inoculum, thus disposing the spores on the surface of the agar. When so arranged, the dilution should be such that the liquid quota for each plate should be raised to about 18 drops (*ca.* 0.9 ml.), and the surplus liquid, after flooding, should be absorbed with sterile filter pads. If the spores are

recognizable, when thus spread on the surface, germination and early transfers of individual spores may be made. A modification of the above is to smear separate drops of the inoculum on areas marked from below, so that observation may be simplified.

In any preparation of inocula for isolation series, every consideration should be given in advance to the promotion of spore formation of the specimen. Commonly, this is attained by a pretreatment which permits the control of moisture and temperature. Adequately moist material in slowly ventilated vessels at a fairly uniform temperature, and with some control of light, are conditions easily available to all.

In the case of many fungi growing upon herbaceous or woody plants, with spores produced in densely stromatic areas or within pycnidia, the mycologist often has access to spores in mass. With favorable moisture, the spores may be exuded as gelatinous horns, as in the chestnut blight fungus; or in masses, as in many anthracnoses. Such material is often nearly free of contaminants.

The forcible discharge of spores from or in sporangia, from perithecia (asci), and from hymenial surfaces has long been known. This field has been closely investigated in recent years by Buller. For present purposes, the interesting mechanism is cited as affording opportunity for catching the spores on sterile slides or cover glasses, excellent material for dilution series, or even for direct transfer to agar slants, with types representative of the three major classes of fungi.

Many special cases arise in the experience of pathologists, both on the plant and on the animal or human side, in which a satisfactory inoculum is difficult to achieve. Often it may be essential to reinoculate the host or a trial host, in the hope of effecting an elimination of secondary organisms. In the establishment of pure cultures of fleshy fungi, it is often reasonable to assume (and has indeed been shown) that direct transfer, aseptically, of small fragments of tissue from a region of growth to prepared media may be the quickest route to satisfactory stock cultures for any practical purpose.

Somewhat analogous to the procedure indicated in the previous paragraph is that often used when an invading fungus grows up through active tissues of the host. Cultures of the corn smut fungus, *Ustilago zeae*, may be obtained readily by direct transfer, after aseptic excision, of tissue containing the fungus. Vascular or cortical parasites (represented, for example, by *Fusarium* sps.) of herbaceous hosts may be transferred directly, if the host tissue is sufficiently large and the exact region of new growth of the invader definitely located. In such cases, usually, surface sterilization by some hypochlorite is an aid.

Procedures with aquatic or certain moist soil fungi belonging to several families of the Phycomycetes, which may be expressed as Saprolegniaceae and allies, are, or may be, essentially dilution techniques. However, the early treatment of the material is often that of casting a pinch of soil (presumably containing some spores) into a small vessel of water, then attempting to "trap" motile spores in this weak soil suspension by throwing in a few seeds of hemp or other fragments of organic matter. Later, if the hemp seeds have served their purpose, it is possible to transfer mycelium and sporangia to fresh containers, so that eventually satisfactory inoculum is obtainable.

It is perhaps apparent that the foregoing brief text could not be intended as a comprehensive guide to the biological isolation of the fungi. The material is offered rather as suggestions respecting current practices, limited, yet sufficiently extensive, it is hoped, to promote interest in a field which is basic to further studies of fungi.

SECTION OF ANTHROPOLOGY

ARCHAEOLOGICAL RESEARCH IN AFGHANISTAN*

By WALTER A. FAIRSERVIS, JR.†

The vast interior region of Asia, the so-called "Inner or Central Asia," has had a strong influence from very ancient times on the high culture centers at its fringes, *i.e.*, India, China, and the Near East. At present, the extent and nature of this influence is largely speculative, because political as well as geographical barriers have effectively impeded thorough scientific investigation of the critical regions. The tendency has been to regard "Central Asia" more as a geographical link, over which passed cultural elements from one "high" culture center to another, than to visualize the area as possessing, within its bounds, centers capable of developing to a high degree certain cultural traits now accredited to other regions. What these traits might be is also speculative, for obvious reasons; but it is felt that such traits as the use of iron, tanning, horse domestication, the compound bow, tailoring, certain bronze-making techniques, and numerous others, are of Central Asian origin.

If "Central Asia" can be understood to include all the region from Manchuria to the Danube in Rumania and from southern Siberia south to the northern mountains of Baluchistan and the Himalaya, it is apparent that there are geographical points within this region which do not conform to the usual, desert-mountain-steppe conception of the area. Such watered regions as the deltas of the Oxus and Jaxartes, the slopes of the northern Altai, the valleys of the Don and the Volga, the slopes of the Ural mountains, and the basin areas such as Seistan and the Tarim are and were suited to agricultural and technological development on a large scale. These areas have either abundant rainfall or a dependable water-table, and the possibilities of supporting an agricultural population outside the nomad pattern are excellent. Failure by archaeology to gain sufficient access to these critical areas, however, has left their true historio-cultural status unknown.

For the reason, then, that these regions cannot be validly generalized as mere bridges between cultures in the face of geographic suitabilities for non-nomadic culture growth, it was and is felt that archaeological research in Central Asia is of great importance for an understanding of the true picture of the development of the high cultures of Asia. With the world political situation preventing access to most of the region concerned, the kingdom of Afghanistan, with its lenient-to-the-West attitude, becomes the obvious area for this necessary research. This is further enhanced when one considers that the Helmand River Valley and the Seistan basin are both within the bounds of Afghanistan.

Whereas the historical records do concern themselves somewhat with certain parts of the Afghan area, *e.g.* Bactria. Drangiana, Arachosia, Sakistan, *etc.*, the prehistory of the country is a complete blank. If however, one considers not only the palaeolithic finds of de Terra, Paterson, and others along the Indus Valley and in Peninsula India, but also the neolithic sites of Iran (Hissar, Sialk, Giyan, *etc.*), Baluchistan (Sur Jangal, Rana Ghundai, Nal, *etc.*), and the Indus Valley (Mohenjo Daro, Haruppa, *etc.*), with their tantalizing traces in painted pottery and other artifacts of cultural inter-relationships, then Afghanistan (and especially Seistan) becomes critically important.

It was with these problems in mind that the First Afghan Expedition of the American Museum of Natural History went to Afghanistan. The initial enterprise was carried out as a survey, with a view not only to test the original theories, but to prepare the way for possible large scale excavation next season. The survey had three prin-

* This paper, illustrated with standard and Kodachrome slides, was presented at the meeting of the Section, February 27, 1950.

† Department of Anthropology, American Museum of Natural History, New York.

cipal aspects, which are described in the following paragraphs.

The first was a geological investigation of the Helmand River Valley along a 200-mile stretch divided into halves at the Girishk line. This was supplemented by excursions into the Dasht-i-Margo, west of the Helmand. No glacial terracing was found in the river valley, but tremendous layers of boulder conglomerate were found overlying a reddish sandstone. This sandstone was found free of the boulder conglomerate some twenty miles into the desert. The age of these formations cannot as yet be ascertained, but the implication of the studies made by Fox and others in Central Afghanistan indicates that the sandstone is Cretaceous or Early Tertiary and that the boulder conglomerates are of later age but probably not Pleistocene. The extent of these gravels some 20 miles on either side of the present river valley indicates that in Tertiary times the Helmand had cut a much larger valley, which was filled in with the gravels resulting from the erosion of the still growing Koh-i-Baga ranges. The present Helmand, confined in its channel, has cut down into this gravel bed to the sandstone base. Overlying this sandstone base is a modern floodplain which is extensively cultivated by the present-day Afghans.

The lack of glacial evidence in this area indicates that the mountains to the south and east prevented moisture deposition on the southern slopes of the Koh-i-Baba and thus impeded the growth of mountain glaciers. This ice-free region may have been, as a consequence, an area suitable for the existence of man and animal. However, no artifacts of Palaeolithic time have yet been recovered.

The second aspect of the expedition's work was the concentration on the locating of sites of the so-called "Chalcolithic" period. These usually take the form of tells. In Seistan, over 35 tells were located and the surface collections made at each of them, while predominantly of Islamic types, have revealed at least one prehistoric site. The climatic conditions encountered prevented a wide search of the area, but it is certain that there are a number of these sites in Afghan Seistan just as there are in Persian Seistan.

In the Quetta Valley, just south of Kohjak Pass, a tell was visited that contained sherds similar to those of Stein's sites at Dabar-Kōt, *i. e.*, a polychrome ware of red and black on red with geometric designs of triangles and lozenges. This valley has a number of large tells scattered here and there which, with the exception of those visited by Piggott in the immediate vicinity of Quetta, are unreported. The promise for future results in this region is therefore very good.

The third aspect of the expedition's work involved a number of small projects which may open the way for full study in the future:

(1) The discovery and photographing of a large number of petroglyphs, which are found wide-spread over the country. On the whole, these are similar to those reported by the Russians from the Pamirs.

(2) The initiating of an anthropological study of the Afghan people. This involved observation, measurement, and photography. Over 50 subjects were studied, and it is hoped that this series may be continued in the future by a physical anthropologist.

(3) A brief visit to the Islamic city of Peshawarun, which lies deserted in Seistan. This is one of a large number of such places in the Seistan basin. It was visited by Goldschmidt, McMahon, Hackin, and Ghirshman but was only briefly reported. The interest to the First Afghan Expedition lay in its extraordinary demonstration of the failure of the water-table in the area. It is an extensive ruin stretching some 6½ miles by 5 miles, with many of the inner walls in excellent preservation. A number of wood and other organic specimens were collected in hope that both Carbon 14 and botanical tests may cast more light on this question of water-table shifts in a limited space of time.

The relations of the expedition personnel with the Afghans were of the best. Both the government and the Afghan people were very cooperative, and the hopes for future work are therefore very good.

Our survey is inconclusive, of course, as most surveys are. It is felt, however, that it has crystallized some of the problems sufficiently for future research in Afghanistan to have every chance for maximum accomplishment.

CONFERENCE HELD

SECTION OF BIOLOGY

"THE GROWTH, REPLACEMENT, AND TYPES OF HAIR"

FEBRUARY 10 AND 11, 1950

The Section of Biology held a Conference on "The Growth, Replacement and Types of Hair." Doctor James B. Hamilton, Long Island College of Medicine, Brooklyn, N. Y., and Mr. Amos E. Light, The Wellcome Research Laboratories, Tuckahoe, N. Y., were the Conference Chairmen in charge of the meeting.

The program consisted of the following papers:

Friday, February 10

Morning Session. Chairman, C. P. Leblond, Department of Anatomy, McGill University, Montreal, Canada.

INTRODUCTION AND GENERAL DESCRIPTION

"Role, Significance, and General Structure of Hair and the Piliary System. The basic Histological Structure of Hair, with a brief Comparison to Feathers, Nails, and Other Appendages," C. P. Leblond.

MORPHOLOGY AND DISTRIBUTION OF HAIR IN MAMMALS

"Morphology and Phylogeny of Hair in Mammals," C. R. Noback, Department of Anatomy, College of Physicians and Surgeons, Columbia University, New York, N. Y.

"Patterns and Distribution of Hair in Mammals," Jerome P. Parnell, Department of Anatomy, Long Island College of Medicine, Brooklyn, N. Y.

"Types and Distribution of Hair in Man" S. W. Garn, Department of Anthropology, Harvard University, Cambridge, Mass.

NORMAL DEVELOPMENT, GROWTH, AND REPLACEMENT OF HAIR

"Development of the Piliary System and the Replacement of Hair in Mammals," E. O. Butcher, Department of Anatomy, New York University School of Medicine, New York, N. Y.

"The Hair Cycle of the Mouse and Its Importance in the Study of Sequences of Experimental Carcinogenesis," S. B. Wolbach, Children's Hospital, Boston, Mass.

"Mitotic Measurements of the Rate of Proliferation of Epidermis and Associated Structures," C. P. Leblond and W. F. Storey, Department of Anatomy, McGill University School of Medicine, Montreal, Canada.

Afternoon Session. Chairman, Mildred Trotter, Department of Anatomy, Washington University School of Medicine, St. Louis, Missouri.

"Rate of Growth, Replacement, and Life Cycles of Hair in Man," R. J. Myers, Department of Zoology, Colgate University, Hamilton, New York, and James B. Hamilton, Department of Anatomy, Long Island College of Medicine, Brooklyn, New York.

"Changes in Morphology of Hair During Childhood," Mildred Trotter and O. H. Duggins, Jr., Department of Anatomy, Washington University School of Medicine, St. Louis, Missouri.

"Appearance of Adult Patterns of Hair in Man," E. L. Reynolds, Samuel Fels Institute, Antioch College, Yellow Springs, Ohio.

"Quantitative Measurements of a Secondary Sex Character, Axillary Hair," James B. Hamilton, Department of Anatomy, Long

Island College of Medicine, Brooklyn, New York.

"Physiological Role of the Piliary System in Mammals and Its Relation to the Environment," L. P. Harrington, John B. Pierce Foundation, New Haven, Connecticut.

CHEMICAL AND PHYSICAL PROPERTIES OF HAIR AND THEIR RELATIONSHIP TO STRUCTURE

"Chemical Classification of Keratins," R. J. Block, Department of Biochemistry, New York Medical College, Flower and Fifth Avenue Hospital, New York, New York.

"Histochemistry and X-Ray Diffraction Studies," A. Giroud, Department of Histology, University of Paris, Paris, France, and C. P. Leblond, Department of Anatomy, McGill University School of Medicine, Montreal, Canada.

"The Results of X-Ray Diffraction Studies on Keratin Fibers," R. S. Bear and H. Rugo, Department of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts.

Saturday, February 11

Morning Session. Chairman, E. O. Butcher, Department of Anatomy, New York University School of Medicine, New York, New York.

"Polariscopic Observations," S. M. Garn, Department of Anthropology, Harvard University, Cambridge, Massachusetts.

"The Role of the Hydrogen Bond in the Structure of Wool," P. Alexander, Wolsey Ltd., Abbey Park Mills, England.

FACTORS INFLUENCING GROWTH, LOSS, AND REPLACEMENT OF HAIR

"Inheritance," L. W. Thigpen, Baptist Memorial Hospital, Memphis, Tennessee.

"Minute Structure of Hair and Its Correlation with Morphology, Physiology, and Behavior," C. E. Keeler, Georgia State College for Women, Milledgeville, Georgia.

"Nutrition," M. L. Morris, Raritan Hospital for Animals, New Brunswick, New Jersey.

"The Effect of Folic Acid Antagonists on Hair Growth," Sidney Farber, Children's Medical Center, Boston, Massachusetts.

"Irradiation," F. P. Ellinger, U. S. Naval Medical Research Institute, Bethesda, Maryland.

"Effects Produced by Atomic Bomb," A. A. Liebow, Department of Pathology, Yale University School of Medicine, New Haven, Connecticut.

"The Relationship of the Adrenal Cortex, Pituitary, and Thyroid Glands to the Growth of Hair," B. L. Baker, Department of Anatomy, University of Michigan School of Medicine, Ann Arbor, Michigan.

"Testes and Ovaries," G. Van Wagenen and W. U. Gardner, Departments of Obstetrics and Anatomy, Yale University School of Medicine, New Haven, Connecticut.

LOSS AND FAILURE OF GROWTH OF HAIR

Afternoon Session. Chairman, Marion Sulzberger, College of Physicians and Surgeons, Columbia University, New York, New York.

"Shedding of Hair in Mammals as a Normal Occurrence," L. W. Thigpen, Baptist Memorial Hospital, Memphis, Tennessee.

"Patterned Loss of Hair in Man. Incidence and Types," James B. Hamilton, Department of Anatomy, Long Island College of Medicine, Brooklyn, New York.

"Patterned Loss of Hair in Man. Pathogenesis and Prognosis," Amos E. Light, The Wellcome Research Laboratories, Tuckahoe, New York.

"The Non-Patterned Alopecias (Alopecia areata, Congenital Alopecia, Febrile States, *etc.*)," C. L. Laymon, Division of Dermatology, University of Minnesota, Minneapolis, Minnesota.

EXCESSIVE DEVELOPMENT OF THE PILIARY SYSTEM

"Hirsutism," G. W. Bissell, Department of Medicine, University of Buffalo School of Medicine, Buffalo, New York.

"Role in Cancer of the Skin," N. C. Foot, Department of Pathology, Cornell University, Ithaca, New York.

"Round Table Discussion of the Piliary System from the Standpoint of Therapeutics"—*Chairman:* Marion Sulzberger, College of Physicians and Surgeons, Columbia University, New York, New York.

1. "Use of Physical and Chemical Agents to Promote Growth of Hair," Marion Sulzberger.

2. "Irradiation," E. M. Uhlmann, Michael Reese Hospital, Chicago, Illinois.

3. "Treatment of Fungus Infections, Baldness, Hirsutism, and Other Pathological States," S. Rothman, Department of Dermatology, University of Chicago, Chicago, Illinois.

4. "Dangers Involved in the Waving of Hair and in the Use of Cosmetics and Dyes," J. Downing, Boston, Massachusetts.

5. "Medicolegal Aspects," B. M. Vance, Office of Chief Medical Examiner, New York Department of Health, New York, New York.

The Section of Biology provides conferences for active workers in the special fields of Biology.

Attendance is limited to those invited to participate in these conferences and to interested members of the Academy.

NEW MEMBERS

Elected February 23, 1950

LIFE MEMBERSHIP

Atlas, Leon T., M.D., Viruses. Surgeon, U. S. Public Health Service, Bethesda, Md.

Blood, Benjamin D., D.V.M., Veterinary Public Health. Chief of Section, Pan American Sanitary Bureau, World Health Organization, Washington, D. C.

Orr, Paul R., Ph.D., Physiology. Associate Professor of Biology, Brooklyn College, Brooklyn, N. Y.

Thurston, Jack T., Ph.D., Chemistry. Director, Research Division, American Cyanamid Co., Stamford, Conn.

SUSTAINING MEMBERSHIP

Gradwohl, R. B. H., M.D., Clinical Pathology. Director, Gradwohl Laboratories, St. Louis, Mo.

Lecher, Hans Z., Ph.D., Organic Chemistry. Director of Research, Calco Chemical Division, American Cyanamid Co., Bound Brook, N. J.

Levin, Ezra, M.S., Biochemistry. President, VioBin Corporation, Monticello, Ill.

Wengraf, Fritz, M.D., Psychiatry, Psychology. Psychiatrist, Beth Israel Hospital, New York, N. Y.

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Andersen, Bjorn, M.S., Chemistry, Physics, Chemical Engineering. Director, Central Research and Development, Celanese Corporation of America, Summit, N. J.

App, Frank, Ph.D., Biological Sciences, Economics. Director, Research and Development, Seabrook Farms Co., Bridgeton, N. J.

Appleby, Douglas C., Jr., B.S., Medical Research. Research Assistant, Department of Zoology, Syracuse University, Syracuse, N. Y.

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Baselt, Frederick C., E.E., Food Preservation, Public Health. Assistant Director of Research American Can Co., New York, N. Y.

Beckmann, Albert J., M.D., Pediatrics. Research Associate, School of Public Health, Columbia University, New York, N. Y.

Bernath, Andrew K., M.D., Cardiology, Mental Diseases. Practicing Physician, New York, N. Y.

Berry, Lorenzo R., M.D., Biological Research, Lung Tumors. Army Medical Corps, Washington, D. C.

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Colville, Kenneth I., B.S., Pharmacology. Student, School of Medicine, Yale University, New Haven, Conn.

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Cragoe, Edward J., Jr., Ph.D., Organic Chemistry. Research Associate, Sharp and Dohme, Glenolden, Pa.

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Curtis, Henry Avery, B.S., Micropaleontology. In Charge, Department of Geology, Hofstra College, Hempstead, L. I., N. Y.

Curtis, Jack M., Ph.D., Endocrinology, Pharmacology. Chief, Sex Hormone Section, Food and Drug Administration, Washington, D. C.

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Donahue, J. Kenneth, Ph.D., Biology. Chairman, Department of Biology, Utica College, Utica, N. Y.

Doob, Dorothy, Ed.Dr., Speech, Psychology. Instructor, Department of Speech, Hunter College, New York, N. Y.

Drobeck, Hans Peter, B. S., Zoology, Parasitology. Graduate Assistant, Department of Zoology, Syracuse University, Syracuse, N. Y.

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Eddy, C. O., Ph.D., Chemical Control of Insect and Insect Diseases. Director of Research, Niagara Chemical Division, Food Machinery Corp., Middleport, N. Y.

Edwards, Charles, M. A., Biophysics. Graduate Student, Johns Hopkins University, Baltimore, Md.

Ellenberg, Max, M.D., Internal Medicine. Senior Clinical Assistant, Mt. Sinai Hospital, New York,

Engstrom, Arne W., M.D., Biophysics, Biochemistry. Assistant Professor, Department of Cell Research, Karolinska Institutet, Stockholm, Sweden.

Erikson, G. L., Physics, Chemistry. Executive Vice-President, Braden Sutphin Ink Co., Cleveland, Ohio.

Featherstone, Robert M., Ph.D., Pharmacology. Associate Professor, Department of Pharmacology, State University of Iowa, Iowa City, Iowa.

Feldman, P. J., D.D.S., Dentistry. Oral Surgeon, Mt. Vernon, N. Y.

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